Zooplankton Grazing Studies

Report 4.4.2

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ZOOPLANKTON PHYTOPLANKTON INTERACTIONS IN THE SAN JOAQUIN RIVER, CA

by

Cyle R. Moon

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DEDICATION

I dedicate this thesis to my wife, Johana. She is the only reason I was able to undertake a Master's degree, and her assistance and support for my academic career are unmatched. I would not be here without her. I also dedicate this work to my daughters, Kaylee and Kendelle. Though they may not realize this at their young ages, they are the reason I strive to be a better scholar, researcher, and person day after day. I love you girls.

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Firstly, I would also like to extend my gratitude to Dr. Gary Litton for giving me, a student from little Merced, this amazing opportunity. If I was the heart of this research, he was the soul. His motivation and resources never wavered. He always made himself available to talk, even if it was irrelevant to our research. I would like to thank Dr. Gary Litton, again, for the unlimited use of his personal research vessel. This was truly what enabled this research to take place. Even when gas prices were painfully high, the boat was ready to go. I would also like to thank Dr. Mark Brunell for always being available to do those early morning sampling trips. His expertise goes unmatched and I am grateful for his assistance in this project. Monica Estiandan, of Dr. Brunell's staff, deserves many thanks for all her work with the zooplankton counts. I would also like to thank Chelsea Spier and all the Ecological Engineering Research Program at University of the Pacific staff for their assistance with my samples for nutrient analysis. Lastly, I would like to thank Dr. Roger Bales for his continual support of me as a student at researcher. He is the reason I first took interest in the Environmental Sciences and his support for me as a student and researcher has given me faith that this is what I should be doing.

Zooplankton-Phytoplankton Interactions in the San Joaquin River, CA

Abstract

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The dynamics of zooplankton and phytoplankton growth and interactions play a significant role in water quality (e.g., pH and dissolved oxygen [DO]) and the available food supply for higher order organisms in the San Joaquin River Delta. Algae have been shown to significantly impact DO concentrations in the Deep Water Ship Channel (DWSC) of the San Joaquin River (SJR) estuary. Zooplankton grazing is one of the important mechanisms that influence the fate and spatial distribution of algae, and therefore, may contribute to DO deficits that adversely impact aquatic habitat and salmonid migration in the SJR estuary. Numerical water quality models developed to simulate and predict dissolved oxygen in the SJR rely on mathematical algorithms that link chemical and biological mechanisms. Due to the complexity of natural systems, calibrating these models is challenging and often requires independent investigations to estimate input parameters, such as zooplankton grazing and algal growth rates. This investigation explored the applicability of three methods to quantify the rates that

zooplankton graze on algae populations in the SJR. Zooplankton grazing studies were performed in the DWSC of the SJR from June 2012 through July 2013. Light and dark bottle microcosm studies using the dilution method, the food-removal method, and the grazer concentration method were tested. A modified microcosm approach similar to the grazer concentration method was developed that yielded changes in chlorophyll *a* concentrations that were sufficient to separate zooplankton grazing from algal growth and respiration. Microcosms contained zooplankton concentrations that were up to 30 times higher than natural, background levels. Zooplankton grazing rates were consistent in both magnitude and variability with literature values reported for other waters, ranging from 0.295-3.404-$m^3gC^{-1}d^{-1}$ and 0.006-1.413-$m^3gC^{-1}d^{-1}$ for light and dark bottle microcosms, respectively.

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C	Clearance rate ($m^3gC^{-1}d^{-1}$)
D	Dilution factor, ratio of unfiltered to filtered water
k	Algal mortality subtracted from algal growth, $k_g - k_r$ (d^{-1})
k_g	Algal growth coefficient (d^{-1})
k_r	Algal decay coefficient (d^{-1})
m	Algal mortality from grazing (d^{-1})
m_v	Phytoplankton viral mortality (d^{-1})
P	Phytoplankton density (µg/L)
P_0	Phytoplankton density before incubation (µg/L)
P_t	Phytoplankton density after incubation (µg/L)
t	Time (hours)
X	Zooplankton density concentration factor (times natural density)
Z	Zooplankton density (µg/L)
Z_0	Zooplankton density before incubation (µg/L)

1. Introduction

During the time period of June-July, 2013, the primary research goal was to develop a suitable method to measure zooplankton grazing in the San Joaquin River (SJR). Firstly, a literature review was done, after which a series of methods were encountered. These methods include the dilution method (Landry and Hassett 1982), the food-removal method (Gauld 1951), and the concentrating grazers method (Capriulo and Carpenter 1980). While each method worked for their respective investigators, the San Joaquin River presents an environment of typically high chlorophyll *a* concentrations and mid-level zooplankton densities that are problematic for quantifying grazing rates. It is well known that not all methods work in all environments, thus, to examine these methods, testing was done on multiple occasions for each (Hansen et al. 1997). In the end, a suitable method was found in a modified version of the concentrating grazers method. The research goals for this project are listed below:

- Develop a method to estimate zooplankton grazing in the SJR
- Use zooplankton grazing rates to identify zooplankton-phytoplankton interactions

Literature Review

Measuring zooplankton grazing rates has importance in understanding algal population dynamics, specifically for application in a predator-prey model. Previous

studies have shown that zooplankton (micro or macro) can consume upwards of 50% of the daily chlorophyll *a* standing stock (Landry and Hassett 1982). The importance of zooplankton is magnified in eutrophic waters in which algal production exceeds typical levels. Urban water bodies are subject to increased nutrient loadings (specifically nitrogen and phosphorous) due to agricultural and human inputs. Studies have shown that eutrophication depletes submerged aquatic vegetation, thus exposing zooplankton to zooplanktivorous fish (Scheffer et al. 1993). In turn, phytoplankton, in the absence of predatory zooplankton, enhance the eutrophication of the water body (Lodi et al. 2011). The San Joaquin River's water quality, although it has improved in some constituents levels (e.g. NH_3) in recent years, still experiences periods of poor trophic state. Evidence of this is in a recent microcystis, a toxic blue-green algae, of which a bloom was observed in the deep water ship channel (DWSC) and other regions of the SJR in the fall of 2012. While zooplankton will generally not consume toxic blue-green algae (Bernardi and Giussani 1990), the bloom of microcystis can be translated into poor trophic state, which is consistent with high phytoplankton populations. Primary phytoplankton removal methods are zooplankton and respiration. Thus, the role of zooplankton in depleting the abundance of phytoplankton is vital to the overall health of the River.

To date, many methods exist to measure zooplankton grazing rates, each one unique in its design. Each method utilizes the direct relationship between phytoplankton and chlorophyll *a*, enabling measurement of chlorophyll *a* concentrations to translate into phytoplankton abundance. The food-removal method utilizes the contrast of chlorophyll *a* growth between microcosms with and without predatory zooplankton (Gauld 1951). Due to its simplicity, the food-removal method is widely used by investigators.

Chlorophyll *a* concentrations can reach values of greater than 100-µg/L, which present an environment in which zooplankton grazing cannot parallel phytoplankton growth.

The dilution method is a commonly used approach for measuring zooplankton grazing. Utilizing the method of grazer dilution with a linear regression fit, the dilution method gives the easiest interpretation of results, with the slope of the linear regression being the zooplankton grazing rate (Landry and Hassett 1982; Landry et al. 1995). While this method has proved feasible for many other studies (Evans et al. 2003; Tijdens et al. 2008; Chen et al. 2012, among others), many of these experiments were in either "perfect" (the meaning of this remains unknown) locations or the results were not ideal. For example, Tijdens et al. (2008) utilized a modified version of the dilution method, designed to measure viral lysis along with zooplankton grazing. While fitting their results with linear regression, the authors never encountered an R^2 of greater than 0.847, and even had a minimum R^2 value of 0.00, while the average R^2 value of their 28 experiments was 0.369. Given the location of their research (shallow, eutrophic, freshwater lake) and its similarities to the San Joaquin River (shallow, slightly eutrophic, freshwater), the dilution method was attempted twice and determined as an unsuitable method. However, the ease of which grazing rates are calculated (linear regression) is convenient.

Ideally, a method to measure zooplankton grazing that allows for amplification of the zooplankton concentration instead of decreasing levels through dilution is needed. Capriulo and Carpenter (1980) developed a method that partly satisfied this criterion by concentrating zooplankton up to 10 times natural densities in order to enhance the micro-zooplankton grazing effect in Long Island Sound, USA. Long Island Sound was subject

to eutrophication effects (O'Shea and Brosnan 2000), and zooplankton densities were likely increased in order to combat accelerated phytoplankton densities. By concentrating zooplankton, rather than diluting, the grazing effect becomes measureable in a system dominated by algal productivity. In the San Joaquin River, where the grazing effect is often masked by phytoplankton growth, this method proved viable.

While grazing methods are inherently straightforward, the underlying dynamics and artifacts are still mostly unknown. For example, much research involves determining when zooplankton feed, in dark hours or light hours. Roman et al. (1988) conducted multiple experiments on marine copepods and found mixed results in terms of when copepods feed. For example, in instances where phytoplankton densities are above 5-µg/L, Roman et al. (1988) found that 90% of copepods would consume their daily rations during dark hours. However, in instances of less abundant phytoplankton, copepods will remain at the surface in dark and light hours in order to meet their daily energy intake requirements. It is understandable that zooplankton prefer to feed in darkness, as they would not be exposed to visually feeding predators. Comparisons of dark and light feeding behaviors are necessary to try to understand zooplankton-phytoplankton interactions.

Since many methods exist to determine grazing rates, grazing rates are often reported with incomparable units (Hansen et al. 1997). For instance, grazing rates reported by Landry and Hassett (1982) are given in units of reciprocal days, or d^{-1}, and daily chlorophyll *a* stock consumption percentages. Capriulo and Carpenter (1980) present grazing rates in terms of chlorophyll *a* removal, expressed as ng chlorophyll *a* removed $animal^{-1}$ h^{-1}. Other studies report zooplankton in carbon equivalents (Hansen et

al. 1997). While conversion between units is possible with measured zooplankton concentrations and an assumed biomass-carbon ratio, many researchers do not present these values, making comparison difficult.

Previous studies involving zooplankton grazing lack a universal approach, as results are dependent on the study methods (Hansen et al. 1997). Evidence is shown in attempts to transfer grazing methods to different environments (e.g. Tijdens et al. 2008) or compare grazing rates between studies. These factors provide motivation for future grazing experiments.

2. Experimentation

Several experiments were conducted from June 2012 through July 2013 using the dilution method, the food removal method, and the concentrating grazers method. Samples were collected from two locations within the San Joaquin River watershed. Study samples were collected from a dock extending to mid-river at Dos Reis County Park, CA or at the Turning Basin of the San Joaquin River Deep Water Ship Channel, located near Stockton, CA, as shown in Figures 1, 2, and 3. Table 1 summarizes the studies performed. Methods utilized for these experiments are outlined in this section.

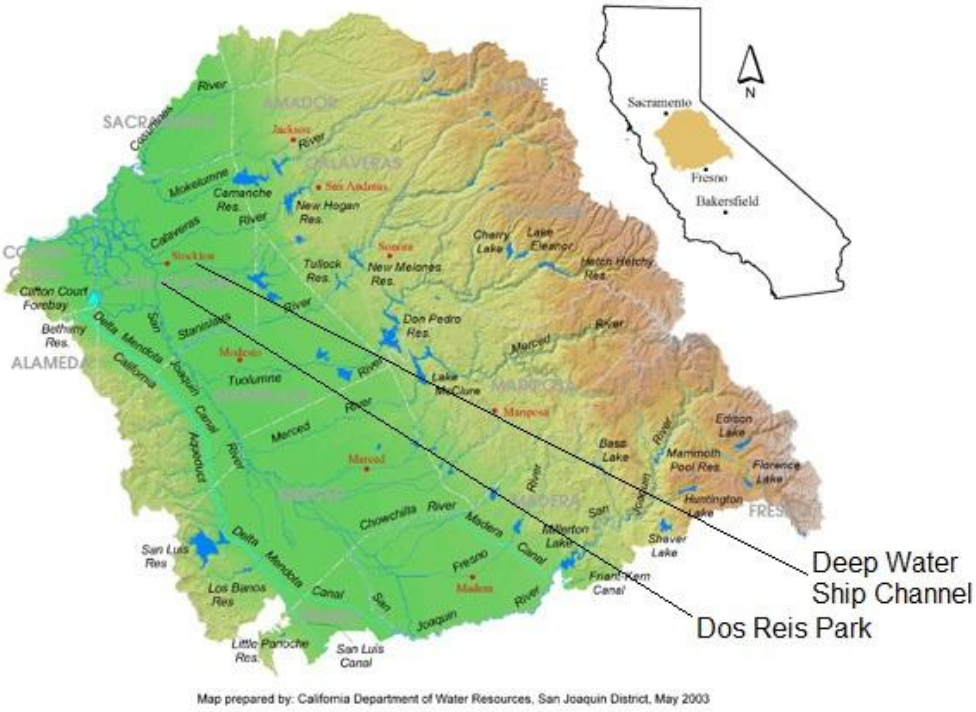

Figure 1. Map of San Joaquin River watershed and sampling locations.

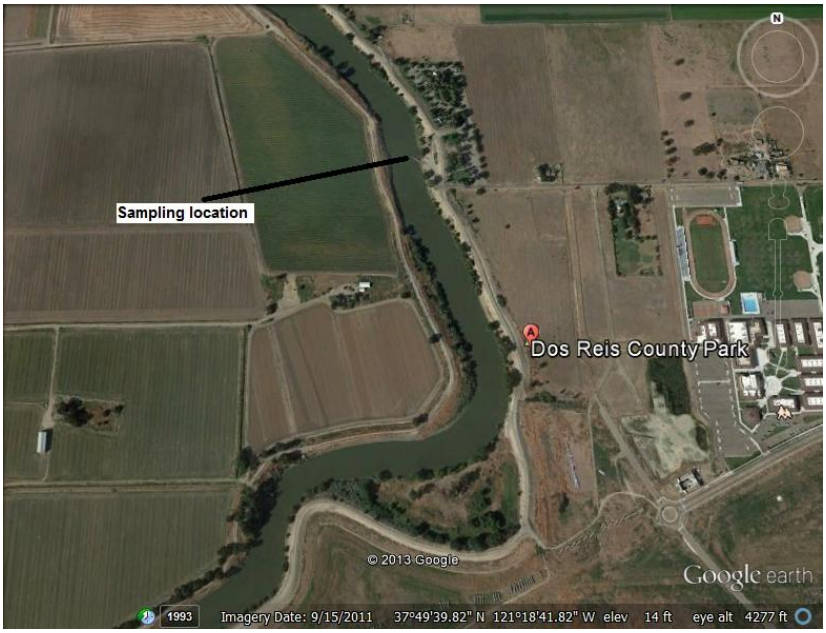

Figure 2. Map showing Dos Reis Park sampling location on the San Joaquin River.

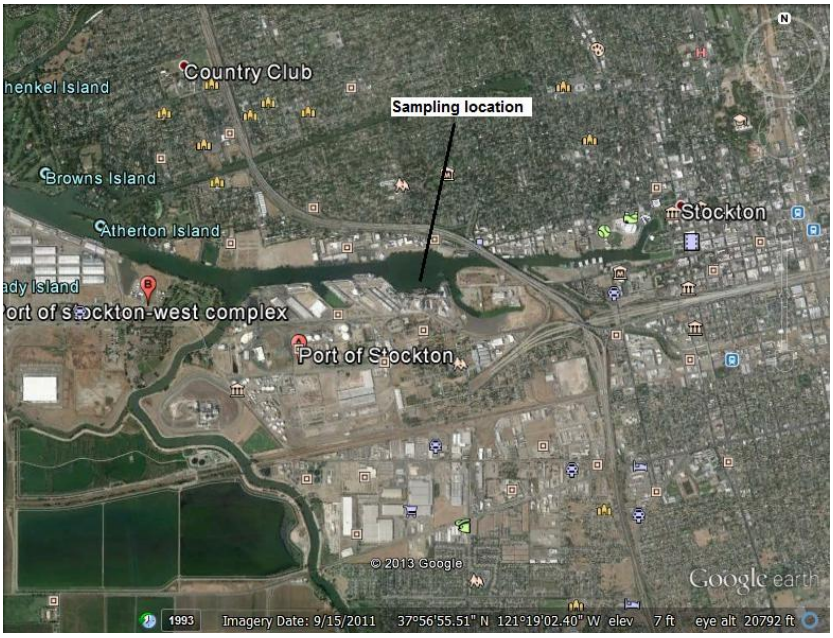

Figure 3. Map showing DWSC sampling location on the San Joaquin River.

These study locations were chosen for several reasons. The Dos Reis Park location was chosen for ease of access. This location was for preliminary experiments only, and with a dock extending to mid-river, this location provided quick sample collection and preparation. The Turning Basin location was chosen based on previous measurements showing consistent phytoplankton and zooplankton populations in this region of the SJR.

Table 1. Summary of zooplankton grazing experiments performed.

Date	Sample Location	Method Used
June 29, 2012	Dos Reis Park	Dilution method (Landry et al., 1982, 1995)
July 17, 2012	Turning Basin	Dilution method (Landry et al., 1982, 1995)
July 31, 2012	Dos Reis Park	Food-removal method (Gauld, 1951)
August 13, 2012	Turning Basin	Food-removal method (Gauld, 1951)
August 20, 2012	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
September 21, 2012	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
October 22, 2012	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
April 18, 2013	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
June 7, 2013	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
June 18, 2013	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)
July 25, 2013	Turning Basin	Concentrated grazers (Modified Capriulo and Carpenter, 1980)

The Dilution Method

One of the more common methods to measure the zooplankton grazing impacts in aquatic systems is the dilution method. This section discusses its origins, as well as how it was used in the San Joaquin River for this study.

Theory. The dilution technique, derived by Landry and others (1982, 1995), establishes a method to estimate the grazing impact of zooplankton on algae using microcosms where the zooplankton density is serially reduce with filtered water. Rates of phytoplankton growth and grazing mortality were estimated from changes in algae concentrations in microcosms incubated with different zooplankton densities. The dilution method approach relies on the following assumptions (Landry and Hassett 1982):

1) The growth of individual phytoplankton is independent of other phytoplankton
2) The likelihood of a phytoplankton cell being consumed is a direct function of the rate at which it encounters consumers with prey cells
3) The phytoplankton density after incubation, P_t, is given by equations (1) and (2) (Franks 2002):

$$\frac{\mathrm{dP}}{\mathrm{dt}} = k_g P - k_r P - CPZ \quad (1)$$

$$P_t = P_0 e^{((k_g - k_r) - m)t} \quad (2)$$

where, k_g is the algal growth constant, $k_r$is the algal decay coefficient, m is the algal mortality associated with grazing (product of the clearance, C and zooplankton density, Z, $m = C * Z$), P_0 is the initial phytoplankton density in the microcosm, and t is time.

Using equation 2, k, a representation of $k_g - k_r$, and m can be found with a minimum of two dilutions. Expressing equation (2) in a linear form:

$$\frac{1}{t} ln \frac{P}{P_0} = k - CZ \quad (3)$$

If microcosms are prepared with some of the zooplankton filtered from the incubated water, then for each microcosm dilution prepared, equation (3) becomes

$$\frac{1}{t} ln \frac{P}{P_0} = k - (CZ_0)D \quad (4)$$

where, D is the fraction of unfiltered water (no zooplankton removed) to total microcosm volume and Z_0 is the zooplankton density prior to dilution. From this equation, the change in phytoplankton density, the left hand side of equation (4), is linearly related to the dilution factor, D. Linear regression analysis gives an equation with slope (CZ_0), the product of the clearance coefficient and the undiluted zooplankton density, and the y-axis intercept, k, the net phytoplankton growth rate.

In many cases, mortality of phytoplankton is not solely caused by zooplankton grazing, but may include other factors, such as respiration, senescence, physical or chemical stresses, and viral lysis or bacterial infection (Evans et al. 2003; Sanderson et al. 2012). Evans et al. (2003) added an additional filtering stage to remove viruses with a 30-kDa membrane to further refine the dilution method by incubating microcosms prepared with zooplankton-free dilution water or virus-free (also zooplankton-free) dilution water. The algal mortality coefficients associated with both viruses and zooplankton grazing were estimated by fitting lines to equations (5) and (6):

$$\frac{1}{t} ln \frac{P}{P_0} = k - (m_v + m) * D_i \ (virus\ and\ grazer\ free) \quad (5)$$

$$\frac{1}{t} ln \frac{P}{P_0} = k - m_v - mD \ (grazer\ free) \quad (6)$$

where, m_v is phytoplankton viral mortality. Through linear regression analysis, the mortality rates due to viral lysis and zooplankton grazing are separated, as illustrated by a hypothetical analysis Figure 4.

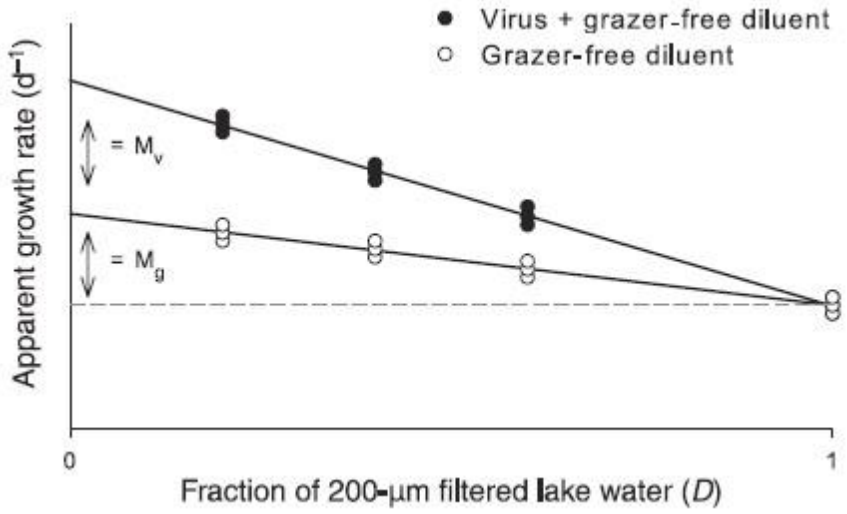

Figure 4. Ideal results from the use of the dilution method (Evans et al. 2003).

Experimental methods. The following section describes the methods used for the dilution method experiments.

Sample collection. One full day of incubation plus 4-5 hours for sampling and preparation was necessary for each microcosm trial. To obtain a single representative sample, water collection was done with a 32-gal plastic drum, and then poured immediately into two 20-L carboys for dark storage. Zooplankton were collected using a

zooplankton trap with an 63-µm plankton filter assembly attached, through which at least 20-L of river water was passed.

Sample preparation. Sample preparation was done onboard the research vessel. Since it is pertinent that samples be processed within four hours of sampling, an onboard filtration apparatus was assembled. Approximately half of the sample water was vacuum filtered through a 20-cm diameter ceramic Büchner funnel equipped with a 20-cm diameter Whatman Reeve Angel grade 230 paper filter (25-30-µm pore size) to remove large particles, such as sediments, in an attempt to speed up the sample preparation process. Due to the high particulate matter present in the SJR, additional filtrations were followed according to Tijdens et al (2008). In this method, a 200-µm filter was used for half of the sample water, removing any large particles and large bodied zooplankton. For our purposes, we intended to study the entire zooplankton population grazing, thus the 200-µm filtration step was not performed on the raw water. The remaining sample water was vacuum filtered through a 18.5-cm diameter Munktel MG550-HA glass fiber filter (1.5-µm pore size), a 47-mm Whatman GF/F filter, then filtered through a Sartorius-Stedim Vivaflow 200 0.2-µm filter cassette to remove any micro-zooplankton. However, the 0.2-µm filter allowed viruses that could affect phytoplankton to pass. Since we intended to study the viral impact on phytoplankton mortality in the San Joaquin River, only 7.5-L of sample water was filtered through the 0.2-µm filter, and the remaining 7.5-L sample was passed through a Sartorius-Stedim Vivaflow 200 30-kDa filter cassette, as shown in Figure 5. This yielded two dilution series, one potentially with viruses and one without. Filtering was performed at pressures less than 30-kPa to reduce injury to aquatic organisms. All equipment was thoroughly cleaned with an Alconox solution

(10-g/L) and rinsed with deionized water multiple times prior to use. Filter cassettes were prepared by passing 1-L of deionized water through each filter before and after experimentation.

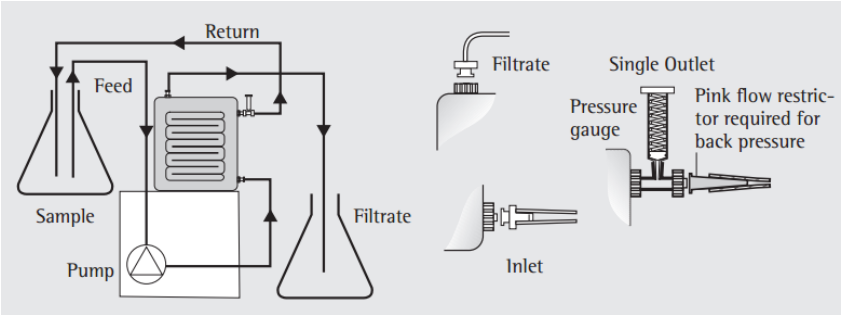

Figure 5. Filtration setup for 30-kDa and 20-µm filter cassettes (Sartorius-Stedim).

Only two dilutions are required for the experiment; however, to produce a statistically permissible linear regression analysis, four dilutions were prepared (Landry and Hassett, 1982). Triplicates of each dilution factor were made in 1.15-L polyethylene bottles. Additional bottles were prepared for control (100% unfiltered water and no added nutrients). The control sample was necessary to observe whether the organism growth was nutrient limited. Each of the three dilution samples had nutrients (nitrate, phosphate) added to them to eliminate a possible growth/grazing limiting factor. Adding macro-nutrients to San Joaquin River water is typically not required due to the high background concentrations; however, addition of $NaNO_3$ and K_2HPO_4 was done as a precautionary measure. Bulk samples for microcosms were made in 4-L plastic containers, and then transferred to the designated sample bottles for incubation. For

nutrient analysis, 120-ml of each sample dilution was extracted. The sample bottles were sealed without air bubbles to prevent any gas/liquid oxygen exchange within the sample. Figure 6 shows a synopsis of the sample preparation order of events.

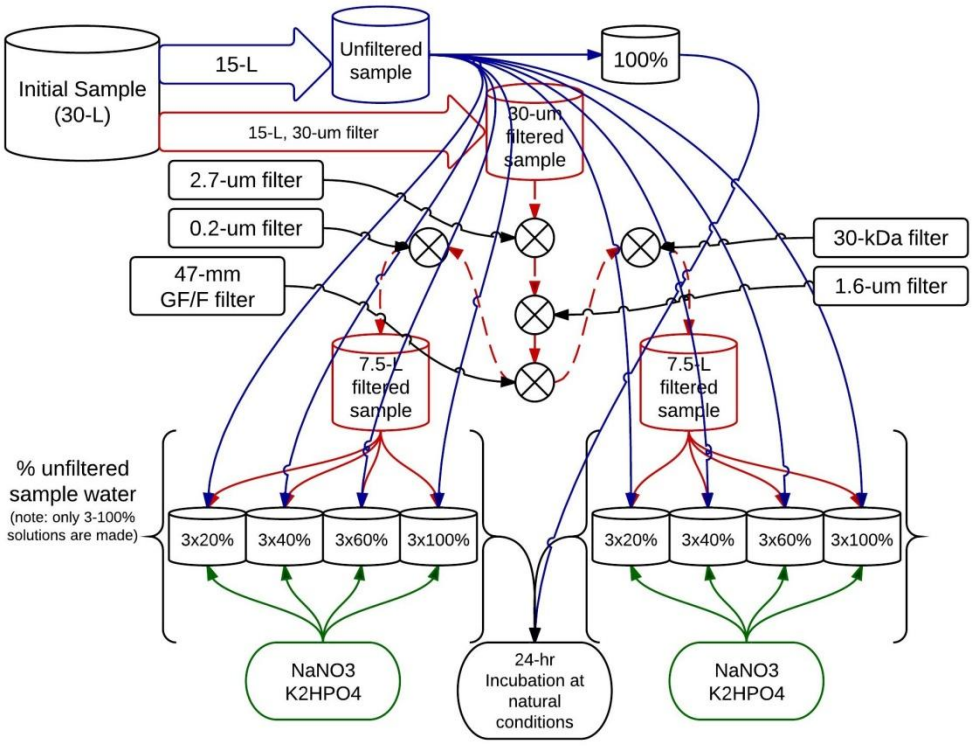

Figure 6. Microcosm preparation method used for the dilution method.

Sample incubation. The prepared sample bottles were incubated in the field for 24 hours. An isolated slough near the San Joaquin River was chosen based on its lack of boating traffic and direct sun exposure without bank of vegetative shading. The samples were tethered to a buoy at water depths of approximately 1-ft.

The Food-Removal Method

A widely used alternative to the dilution method is the food-removal method, developed by Gauld (1951). Microcosms with and without grazers are incubated in darkness. The difference in chlorophyll *a* concentrations at the end of the incubation period yields the grazing rate by subtracting the algal losses associated with decay (microcosms without zooplankton) and directly calculating the algal mortality associated with grazing (m).

Experimental methods. The following section describes the methods used for the food-removal method grazing experiments.

Sample collection. Studies in the San Joaquin River at Dos Reis Country Park, CA (July 31, 2012) and in the Turning Basin (August 13, 2012) were conducted. Water samples were collected from locations with a 32-gal drum. This sample was then divided between 1-L polycarbonate bottles for incubation. Three types of microcosms were prepared: whole water, no zooplankton, and concentrated zooplankton (4X concentrating was performed as an experimental trial). Zooplankton were removed from the collected water using a 63-µm plankton net. Concentrated zooplankton microcosms were prepared from zooplankton captured in the plankton net.

Sample incubation. Samples stored in 1-L polycarbonate bottles were incubated in a dark environmental chamber at 25°C. To evaluate potential settling impacts on grazing, selected microcosms were mixed with a stir plate at low speed throughout incubation. Chlorophyll *a* measurements were performed at time periods of 0, 3, 6, 24, 30, and 54-hr from dedicated microcosms. In the August 13, 2012 study, the 54-hr measurement was excluded.

Modified Grazer Concentration Method

Theory. The grazer concentration method was developed by Capriulo and Carpenter (1980) for field, in-situ measurements of zooplankton grazing. Without concentrating zooplankton, changes in chlorophyll *a* during the incubation period had proven to not be discernible. By concentrating the zooplankton, this method potentially yields greater reductions in chlorophyll *a* and grazing mortality becomes more apparent, as the influence of zooplankton grazing is no longer masked by phytoplankton growth or inherent variability in chlorophyll *a* measurement.

The grazer concentration method was employed with microcosms using an approach similar to the dilution method. Equation (3) serves as the basis; however, instead of assuming that C and Z_0 are function of a dilution factor D (as in the dilution method), in this case we assume that C and Z_0 are functions of a concentration factor, X. Simply, $Z = Z_0X$. Substituting this into equation, the resulting analytical solution is presented in equation (7):

$$\frac{1}{t} ln \frac{P}{P_0} = k - (CZ_0)X \qquad (7)$$

where, X is the zooplankton density concentration factor. The other parameters are defined earlier.

Sample collection. Grazing trials were performed using the concentrating grazers method as stated in Table 1. By August 2012, concentrations of zooplankton and algae were extremely low in the San Joaquin River due to zero net flow conditions that persisted throughout the summer 2012. However, plankton populations remained high in the Turning Basin; therefore, collection of sample water was restricted to this location.

Zooplankton samples used to concentrate the microcosms were taken from mid-depth (~20-ft.), but water samples were collected from the surface.

Microcosms were prepared at concentrations of 0, 1, 7.5, 15, 22.5, and 30 times (X) the background zooplankton density. The 22.5X concentration was added during the September 21, 2012 study to investigate possible saturation feeding occurring at high zooplankton concentrations. Zooplankton samples were collected with a 30-L Shindler-Patalas trap fitted with a 63-μm plankton net, as described previously. The control microcosms (0X) consisted of river water filtered through the plankton net.

Sample preparation and incubation. Microcosms were conducted in 1-L polycarbonate bottles. The concentrated zooplankton stock was well-mixed before the preparation of each microcosm, as was the river water. Prepared microcosms remained in darkness until deployment into the river. Prior to incubation, one to two preparations of each microcosm concentration were reserved for initial chlorophyll *a*, nutrient, and zooplankton measurement. Microcosms were incubated at varying times for the different study dates, to facilitate greater reductions in chlorophyll *a* and get an average grazing response for days with light and dark periods. Table 2 describes the incubation times and locations.

Table 2. Incubation times and locations for studies using the modified concentrated grazers method.

Study Date	Incubation Start Time	Incubation End Time	Total Incubation Time (hours)
August 20, 2012	8/20/12 9:00	8/20/12 15:00	6
September 21, 2012	9/21/12 8:30	9/21/12 15:30	7
October 22, 2012	10/22/12 9:30	10/23/12 7:10	22.5
April 18, 2013	4/18/13 10:00	4/19/12 10:00	24
June 7, 2013	6/7/13 10:00	6/8/13 10:00	24
June 18, 2013	6/18/13 9:40	6/18/13 9:40	24
July 25, 2013	7/25/13 12:30	7/25/13 12:30	24

Figure 7 shows the incubation buoy setup, while Figure 8 shows the incubation location. Samples were incubated at 1-ft. water depths. This location was selected due to infrequent boating traffic, as well as the ability to incubate samples in even sunlight, enabling the experiments to all experience the similar phytoplankton growth capabilities.

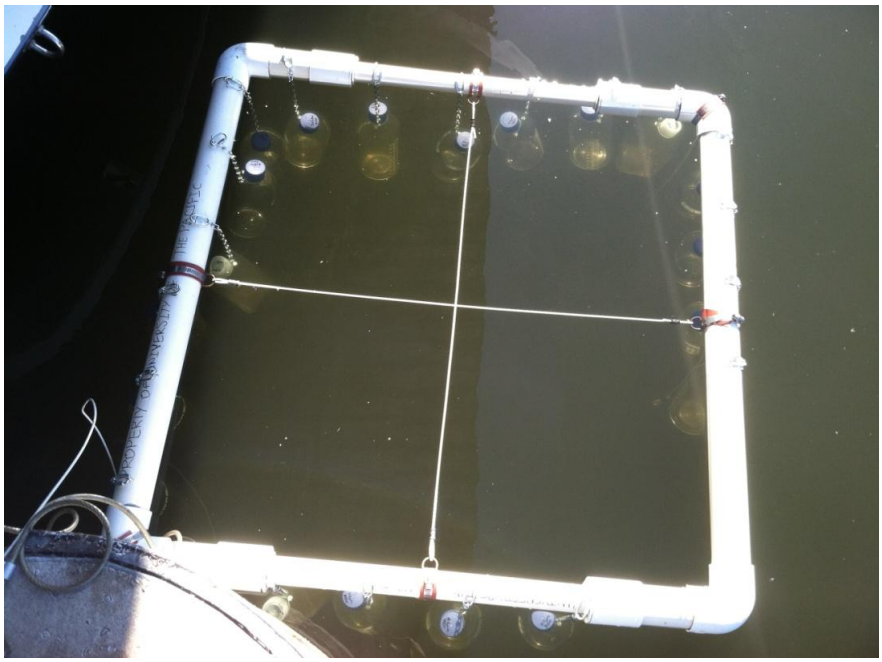

Figure 7. Incubation buoy setup, with suspended microcosm bottles.

Figure 8. Incubation location outside of Village West Marina, Stockton, CA. Incubation buoy is at left-center.

After collection, the samples were immediately placed on ice in a closed cooler, and transported directly to the laboratory for chlorophyll *a* extraction.

Nutrient and Zooplankton Analysis

Prior to the incubation period, subsamples of each microcosm were analyzed for nutrients and zooplankton. Samples for nutrient analysis were immediately placed on ice for preservation. Dissolved nitrate + nitrite nitrogen (NO_3-N + NO_2-N) and total ammonia/ammonium nitrogen (TAN) were quantified using an automated membrane diffusion/conductivity detection method (Carlson 1978; Carlson 1986; Carlson et al. 1990). Dissolved Nitrite (NO_2-N) was measured colormetrically by SM 4500-NO_2 (APHA 2005). Total nitrogen (TN) was determined by the same method from unfiltered sample following persulfate oxidation (Yu et al. 1994). Dissolved ortho-phosphate (PO_4-P) was quantified in filtered samples by the stannous chloride method SM 4500-P.D (APHA 2005). Total phosphorus (TP) was determined on unfiltered sample by persulfate digestion (Yu et al. 1994) and colorimetric determination by the stannous chloride method SM 4500-P.D (APHA 2005). Dissolved silica was analyzed by using a modified Heteropoly Blue molybdosilicate method (modified SM 4500-SiO2 D) (APHA 2005).

To cease organism activity, Lugol's iodine fixative was applied immediately to the samples designated for zooplankton measurement. Zooplankton samples were thoroughly mixed by inversion and a 5-20-mL subsample was taken from each using a Stempel pipette (volume adjusted for sediment amount in sample). The subsamples were

added to a settling apparatus, and settled for 5–20 hrs depending on volume. Prior to settling, 100-µL of 1% rose Bengal dye was added to facilitate counting of zooplankton.

Zooplankton were examined with a Leica DM-IL inverted microscope. Identification of species follows standard texts (Balcer et al. 1984; Chengalath et al. 1971; Pennak 1989; Pontin 1978; Wallace 1991).

During zooplankton counts, the entire chamber floor was examined. For biomass estimates, body measurements were taken from a maximum of twenty individuals of each species using a calibrated ocular Whipple Grid. Conversion of body measurements into biomass follows EPA (2003) publication LG403. Following publication LG403, a minimum of 200 individual organisms are counted for each sample.

Repeat nutrient and zooplankton analysis was done after the incubation period to capture the final respective concentrations of each sample. Zooplankton analysis for samples using the concentrated grazers method was performed before and after the incubation periods. In all cases, zooplankton analysis was done initially, but not for all mixtures.

Chlorophyll *a* Analysis

Microcosms were individually sampled for chlorophyll *a* before and after the incubation period per the Standard Method for measuring chlorophyll and pheophytin *a* (APHA 2005). Subsamples ranging from 250-1000-ml were vacuum filtered through a Whatman 47-mm GF/F filter, then washed with a 10-g/L solution of magnesium carbonate ($MgCO_3$) to prevent chlorophyll *a* decay. Filters were then carefully removed from filter assembly and placed in coin envelopes and frozen until further processing.

Within 28 days of sampling, samples were ground in a 90% acetone, 10% deionized water solution with a glass tissue grinder. Once ground, the filters were placed in 15-ml centrifuge tubes and stored in darkness at 0ºC. Within 24 hours of grinding, samples were centrifuged to separate solid filter debris and processed via spectrophotometer in a 5-cm cell at wavelengths of 750, 664, 647, and 630-nm before acidification, and at wavelengths of 750 and 665-nm after acidification. In cases where microcystis was abundant, samples were pre-screened through a steel mesh (Type 316 stainless steel, 47-mm diameter) to remove any inedible algae (Bernardi and Giussani 1990). The pre-screening was only performed with the June-July, 2013 studies. Tests were done to determine if the chlorophyll *a* addition by these particles was significant. These tests showed that chlorophyll *a* concentrations were amplified by the inedible algae. Chlorophyll *a* concentrations after microcystis removal were used for the grazing rate calculations.

3. Results

The results of the dilution, food-removal, and grazer concentration methods, as applied to the San Joaquin River, are discussed below. Only the grazer concentration method provided measureable clearance rates of algae by zooplankton. However, microcosm experiments performed during the trials in which zooplankton were removed or incubated in darkness provided estimates of algal productivity and decay. Additionally, nutrient sample concentrations for nitrogen, phosphorous, and silica species are presented to establish whether limiting conditions influenced the microcosm experiments.

Dilution Method Results

Results obtained from the June 29, 2012 study at Dos Reis Country Park are shown in Figures 9 and 10. Algal mortality coefficients (m) were 0.0524-d^{-1} and 0.039-d^{-1} for total grazing and viral decay, respectively. Chlorophyll *a* concentrations ranged from 25-µg/L to 132-µg/L. Figure 9 illustrates the phytoplankton growth during the incubation period. All of the microcosms experienced growth; however, the growth is unpredictable in each microcosm, with ranges of chlorophyll growth up to 20-µg/L of chlorophyll *a* for similar microcosms. This variability between microcosms is shown in Figure 9.

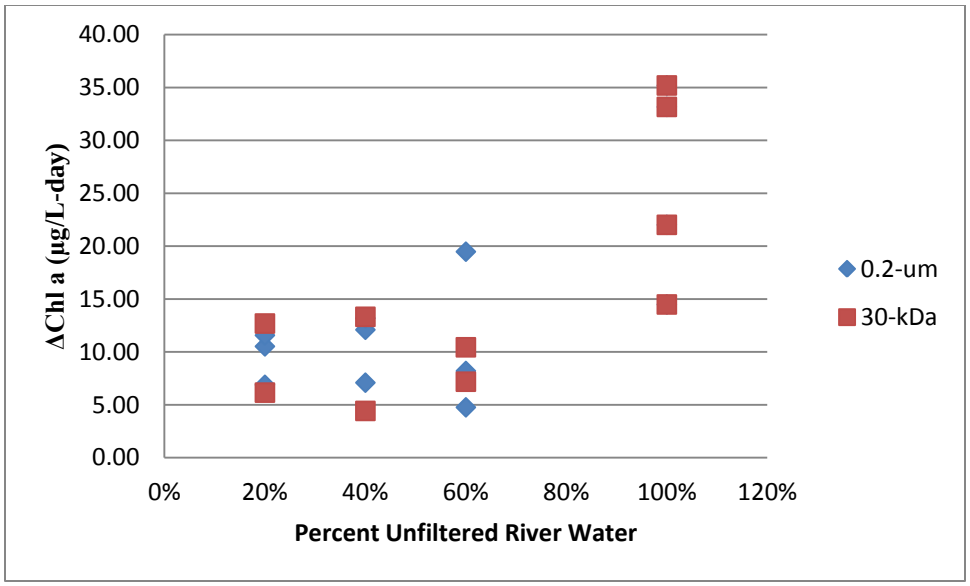

Figure 9. Change in chlorophyll *a* concentrations for each dilution series during the 24 hour incubation period on June 29, 2012

The zooplankton grazing rate, m (units of d^{-1}), could not be measured from linear regression analysis (see equations [5] and [6]) because of poor linear fits and intersecting regression lines (see Figure 10). Efforts to utilize only the filtered zooplankton microcosm data to estimate algal mortality associated with grazing only (m) were unsubstantiated because the correlation coefficients for the linear regression analysis were poor, suggesting that these rates may be unreliable (see Figure 10).

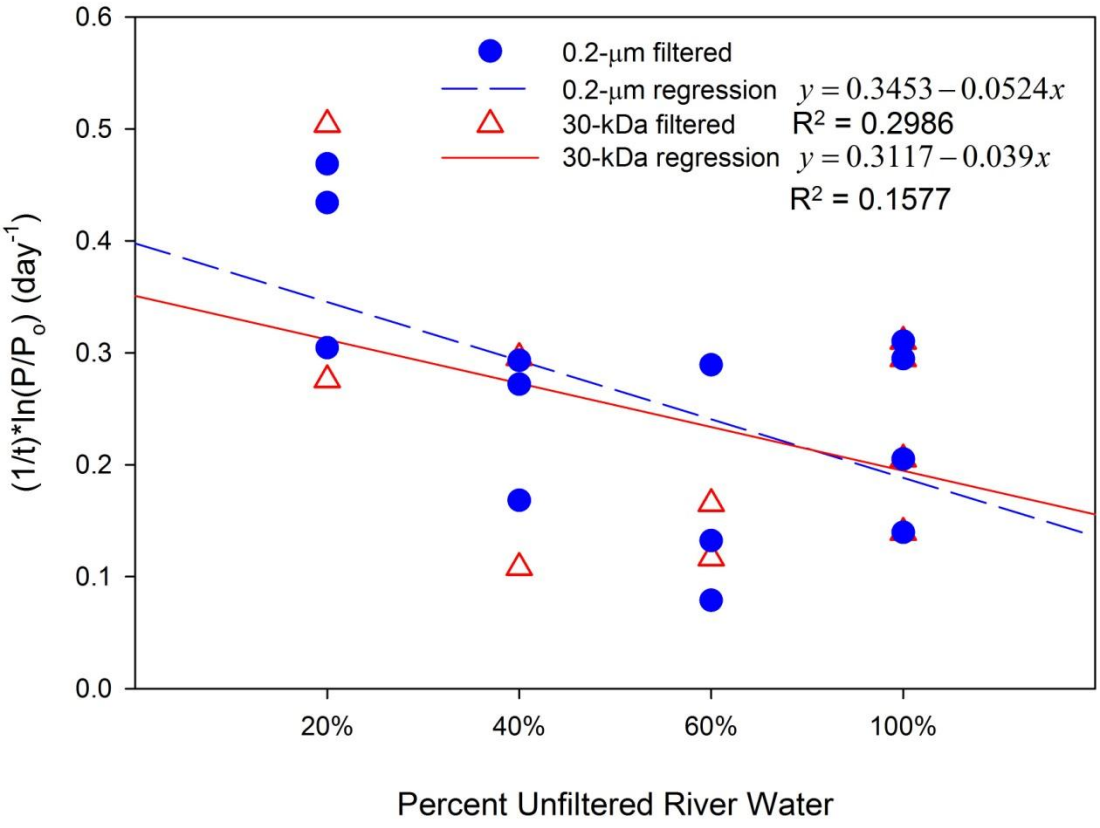

Figure 10. Phytoplankton growth rates via application of the dilution method (Tijdens et al. 2008) to chlorophyll *a* concentrations obtained on June 29, 2012.

Figure 10 shows unexpected results. With this application of the dilution method, the microcosms with and without viruses should follow a pattern similar to that illustrated in Figure 4, with the virus and grazer free microcosms experiencing higher phytoplankton growth rates. However, in this study, the microcosms with and without viruses experienced similar phytoplankton growth rates, in addition to poor linear fits. Results from this experiment are of limited use.

Figure 12 illustrates the results obtained from application of the dilution method on July 17, 2012 at the Turning Basin location of the San Joaquin River. These results were also not able to be analyzed due to poor correlation coefficients and intersecting lines. For both trials, grazing effects appear to be masked by high algal growth rates, as shown in Figure 11.

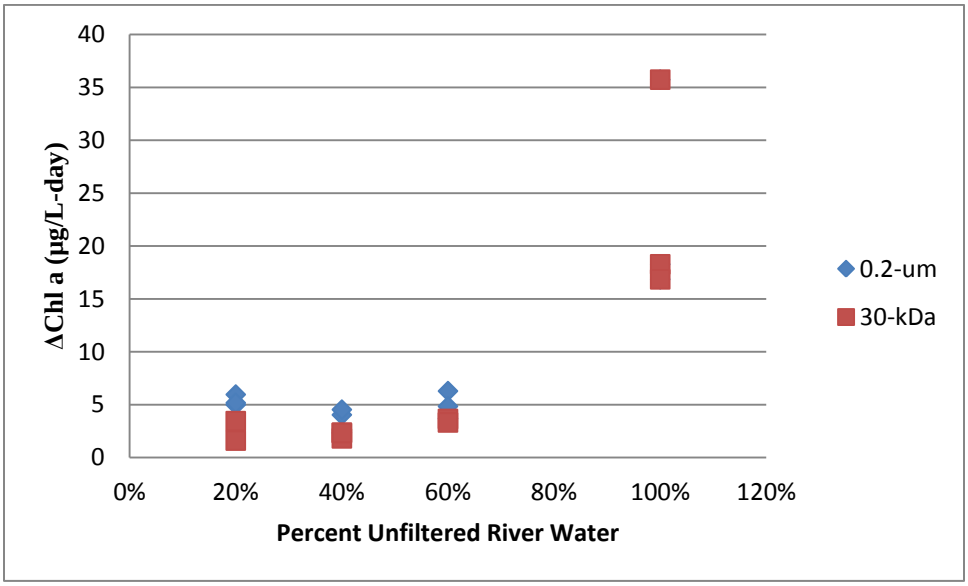

Figure 11. Change in chlorophyll *a* concentrations for each dilution series during the 24 hour incubation period on July 17, 2012.

The poor linear fits and irregularities presented in Figure 10 and 12 have also been observed by other investigators for different water bodies (e.g. Tidjens et al. 2008, where 80% of trials failed to yield measurable grazing rates). Thus, this method appears to be inappropriate for the San Joaquin River environment, even though it was recently

used with success in an Australian lagoon to elucidate the dependence of the grazing rate on algal productivity (Sanderson et al. 2012). However, the June 29 and July 17, 2012 trials did provide phytoplankton productivity rates, which are presented and discussed later.

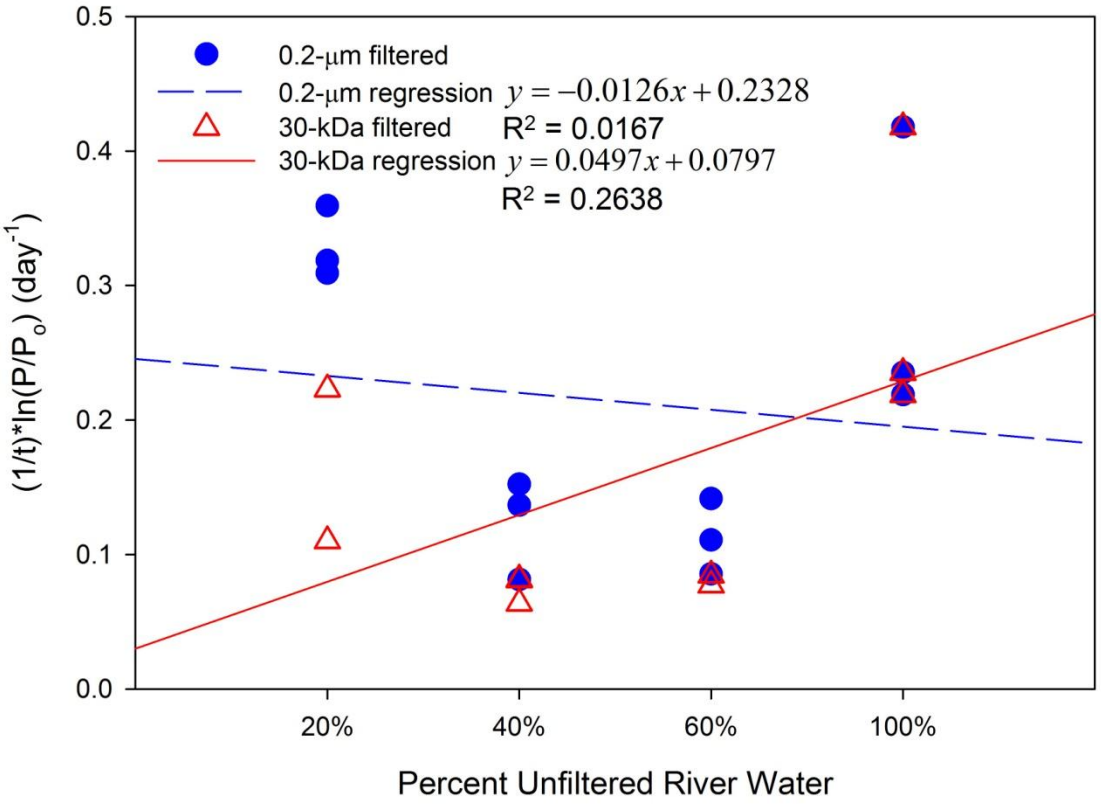

Figure 12. Phytoplankton growth rates via application of the dilution method to chlorophyll *a* concentrations obtained on July 17, 2012.

Zooplankton and nutrient results. Water samples were analyzed for the June 29 and July 17, 2012 grazing experiments. Zooplankton densities of 14.99-µg/L and

200-µg/L were measured for the June 29 and July 17, 2012 experiments, respectively. The low zooplankton densities measured for the June 29, 2012 study may explain the unpredictable results illustrated in Figure 10. With a lack of grazers, the microcosms were dominated by chlorophyll growth (see Figure 9), which was variable as well. Without consistent growth patterns and a lack of grazers, the results obtained for June 29, 2012 were highly variable, and did not yield accurate estimates of zooplankton grazing. The July 17, 2012 results experienced similarly variable chlorophyll growth patterns; however, grazers were abundant in these microcosms.

Nutrient analysis was performed only for the July 17, 2012 study, as the June 29, 2012 study served as a preliminary test of filtration methods. Nutrients were added to the July 17, 2012 microcosms before incubation according to Tijdens et al. (2008), and analysis was only done after the incubation period. Relevant nutrients, nitrate and phosphate, were measured at concentrations of 0.821-mg/L and 0.199-mg/L, respectively, indicating a nutrient limitation was nonexistent for this grazing study (see Table 5).

Food-Removal Method Results

Results from the July 31, 2012 and August 13, 2012 studies conducted using the food-removal method are presented in Figures 13 and 14, respectively. Figures 13 and 14 show chlorophyll *a* concentrations for the various microcosms (mixed with grazers, unmixed with grazers, without grazers) at different measurement intervals. Figure 13 shows a consistent decline in chlorophyll *a* concentration with incubation time

(experiments were performed in complete darkness); however, there was little difference in chlorophyll *a* removal rate (similar line slopes) between samples with and without zooplankton. In addition, mixing did not appear to influence the rate of decrease in chlorophyll *a* concentration, as the lines paralleled one another. The July 31, 2012 study was not analyzed for zooplankton or nutrients, as it served as preliminary study.

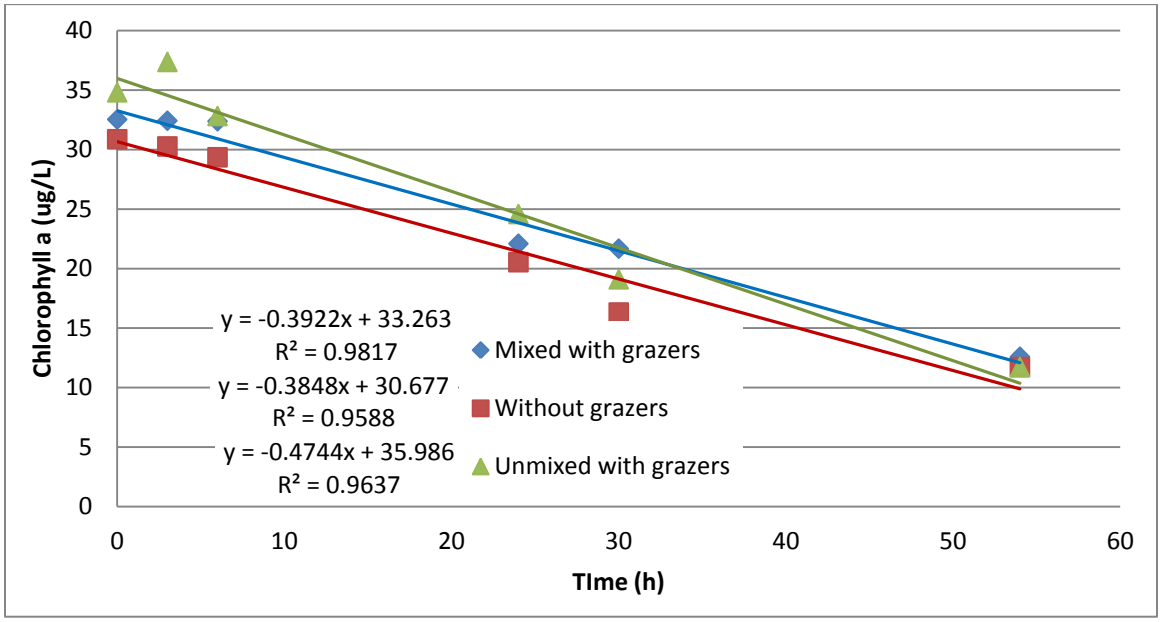

Figure 13. Average chlorophyll *a* concentrations versus time with linear fits for samples obtained on July 31, 2012.

Figure 14 shows the results from the application of the food-removal method to samples collected at the Turning Basin location on August 13, 2012. The difference in chlorophyll *a* concentrations between the various microcosms is unnoticeable, implying

that zooplankton grazing was insignificant in chlorophyll *a* removal. As the incubation time increases, a minor separation occurs; however, at a 30 hour incubation period, one would expect the zooplankton grazing impact to be greater. Sample analyses revealed no zooplankton were present in the microcosms, explaining the unnoticeable grazing effect. This is illustrated through the similar line slopes between microcosms with and without grazers. Thus, the phytoplankton mortality was due entirely to respiration.

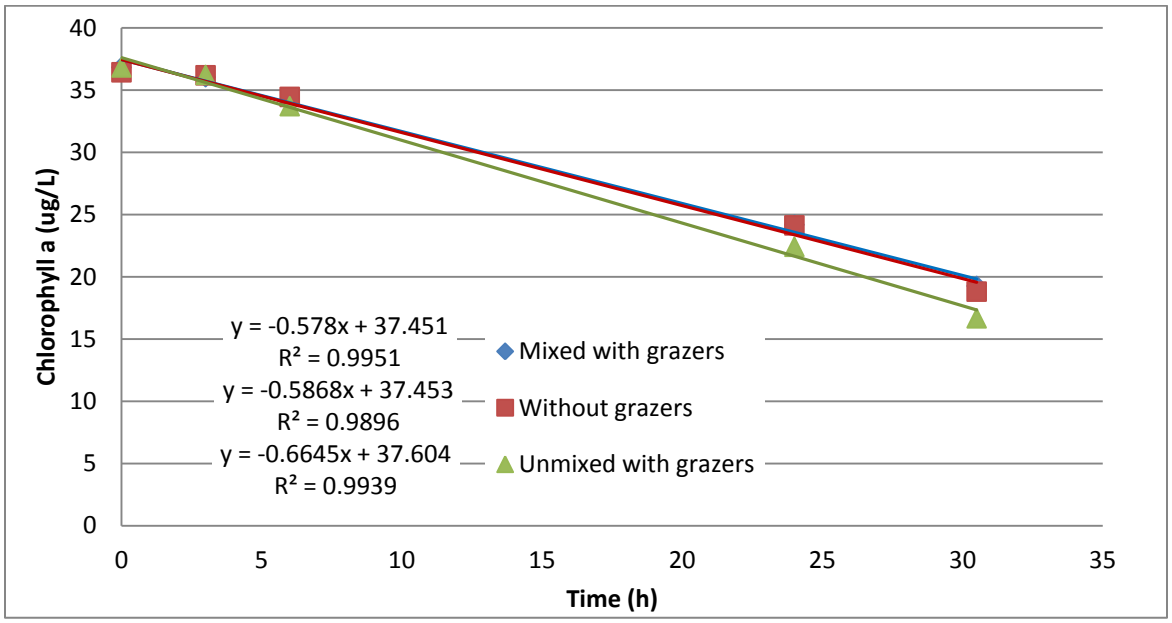

Figure 14. Average chlorophyll *a* concentrations versus time with linear fits for samples obtained on August 13, 2012.

In the July 31 and August 13, 2012 food-removal studies, zooplankton grazing was immeasurable. While previous studies show highly variable phytoplankton

populations, zooplankton populations are also variable. Because zooplankton densities remain unknown until after experimentation, grazing experiments often do not follow predicted patterns. In cases where zooplankton are limited or nonexistent, grazing experiments reveal no information regarding grazer induced phytoplankton mortality. This is a drawback of this method, and grazing methods in general, in areas where zooplankton populations are highly variable.

Modified Grazer Concentration Method Results

Zooplankton results. Zooplankton counts were completed for the grazing experiments completed from August, 2012 through July, 2013. While only the 2013 studies included zooplankton concentrations measured at each microcosm concentration, the same concentration method was used for all studies. Figures 15-18 illustrate the accuracy of the process utilized to concentrate the zooplankton and the accuracy of the zooplankton analysis method. With a minimum R^2 value of 0.9634, it is fair to say that the method used to concentrate zooplankton in the individual microcosms is sufficient.

Figures 15-18 show total zooplankton (all species) densities, obtained from zooplankton counts, as a function of designed zooplankton concentration. While the initial zooplankton densities match the designed zooplankton concentrations, the final zooplankton concentrations do not exhibit this pattern. In all studies, the zooplankton densities decrease for the higher zooplankton concentrations (15, 22.5 and 30X). This was not expected; however, it could help to explain grazing results presented later in this

chapter. A summary of the linear regression fits to initial zooplankton densities is presented in Table 3.

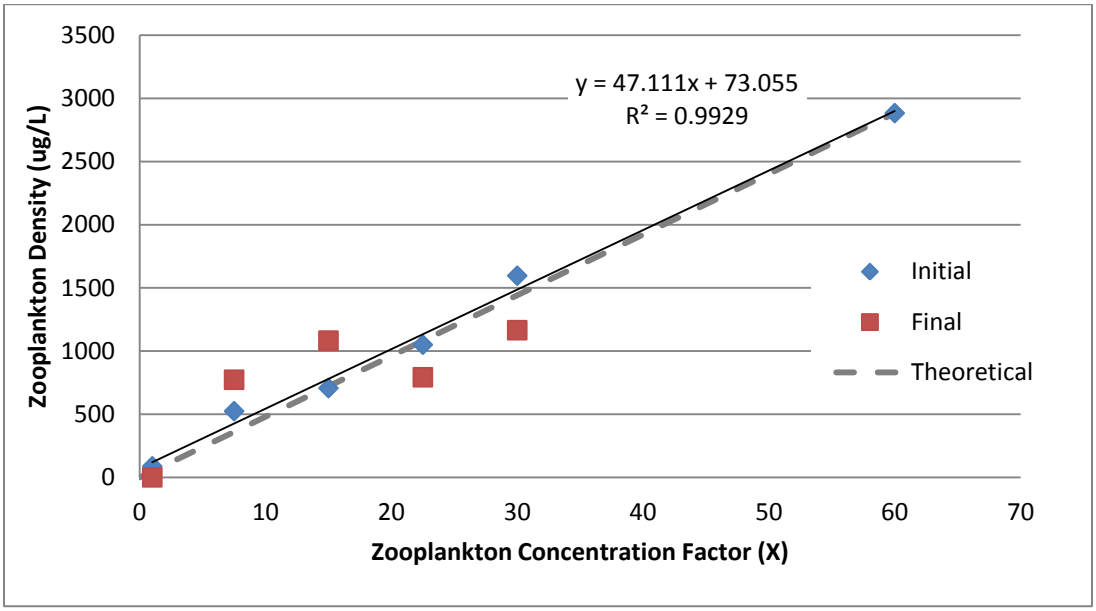

Figure 15. Total zooplankton density versus designed concentration factor (X) with linear fit for the April 18, 2013 grazing study.

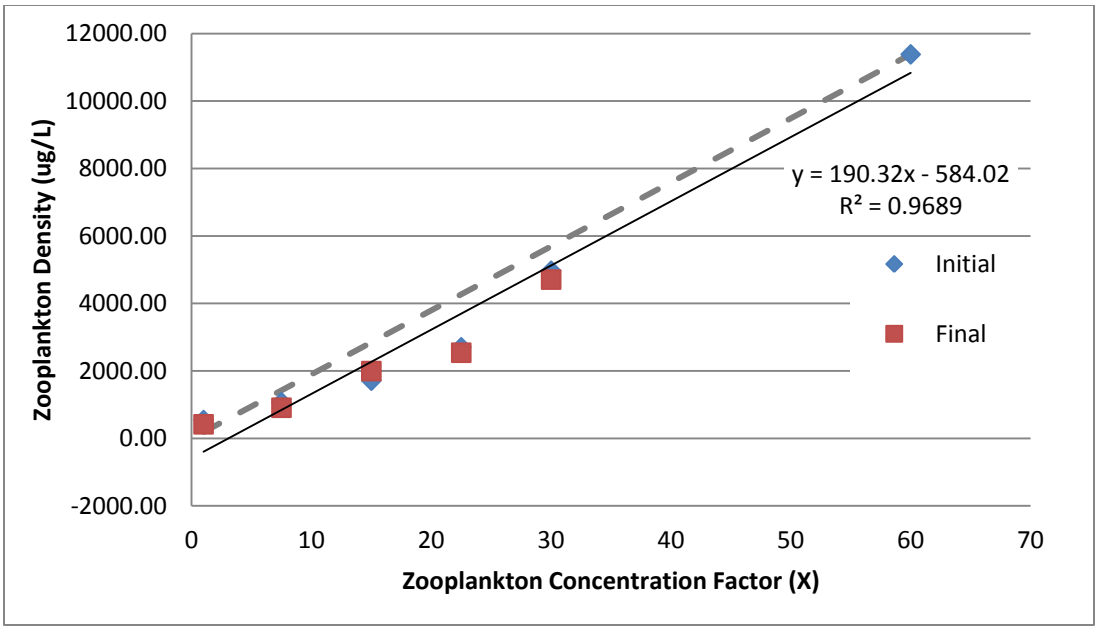

Figure 16. Total zooplankton density versus designed concentration factor (X) with linear fit for the June 7, 2013 grazing study.

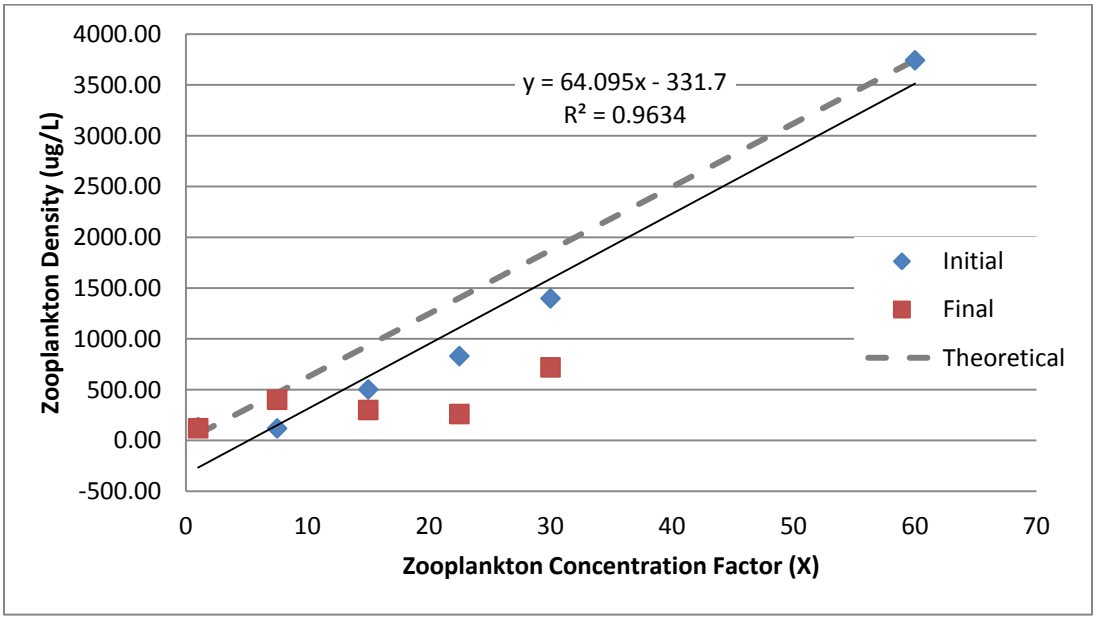

Figure 17. Total zooplankton density versus designed concentration factor (X) with linear fit for the June 18, 2013 grazing study.

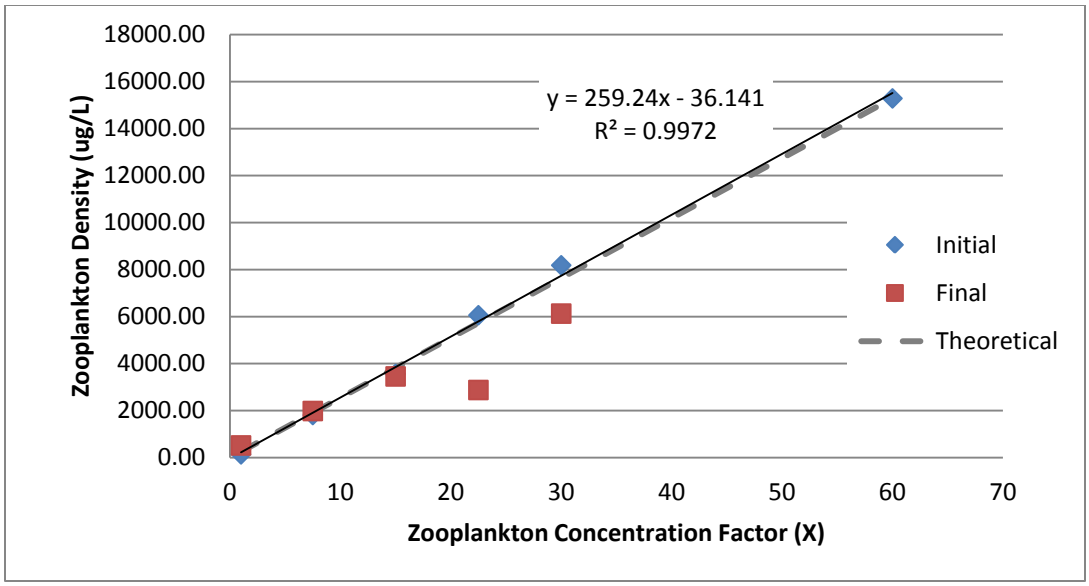

Figure 18. Total zooplankton density versus designed concentration factor (X) with linear fit for the July 25, 2013 grazing study.

Table 3. Summary of initial zooplankton density linear regression fits.

Date	Initial R^2
April 18, 2013	0.9929
June 7, 2013	0.9689
June 18, 2013	0.9634
July 25, 2013	0.9972

Chlorophyll a results. Figures 19-21 present absorbance ratios for wavelengths of 664-nm (before acidification) and 665-nm (after acidification). The ratio of these wavelengths is helpful in determining the health of the phytoplankton within the water sample. Essentially, a high ratio (typically around 1.70) is associated with a healthy phytoplankton population. The analysis in Figure 19 serves as proof that the sample preparation method does not harm the phytoplankton within the microcosms, as most

samples exhibit ratios between 1.50 and 1.75, with a few outliers. These outliers are likely associated with microcystis contamination in the samples, as these particles were observed during this study. Efforts to remove these particles were undertaken; however, contamination was still possible.

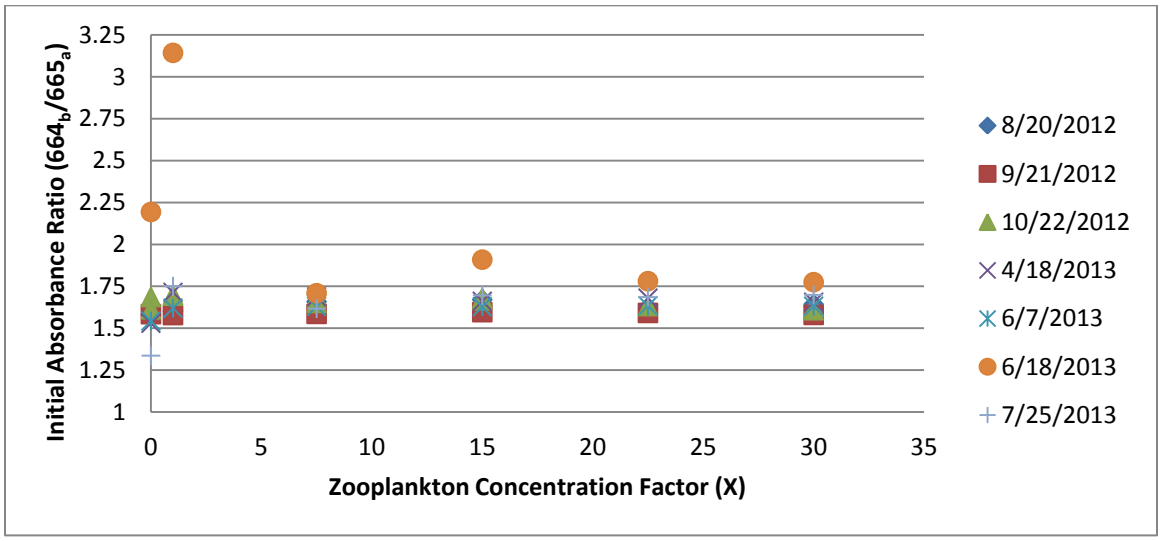

Figure 19. Absorbance ratios for initial chlorophyll samples using the concentrated grazers method.

Figures 20 and 21 show the absorbance ratios after the incubation periods for both light and dark bottle microcosms, illustrating the stresses placed on phytoplankton within the microcosms during the incubation period. Figure 20 shows that the phytoplankton populations within the light microcosms were typically healthy, as all most ratios were between 1.5 and 2.0.

Figure 21 shows that the health of the phytoplankton populations within the dark microcosms was more variable than that of the light microcosms. The range of absorbance ratios was 1.40-1.95. However, this range of absorbance ratios is still associated with healthy phytoplankton populations.

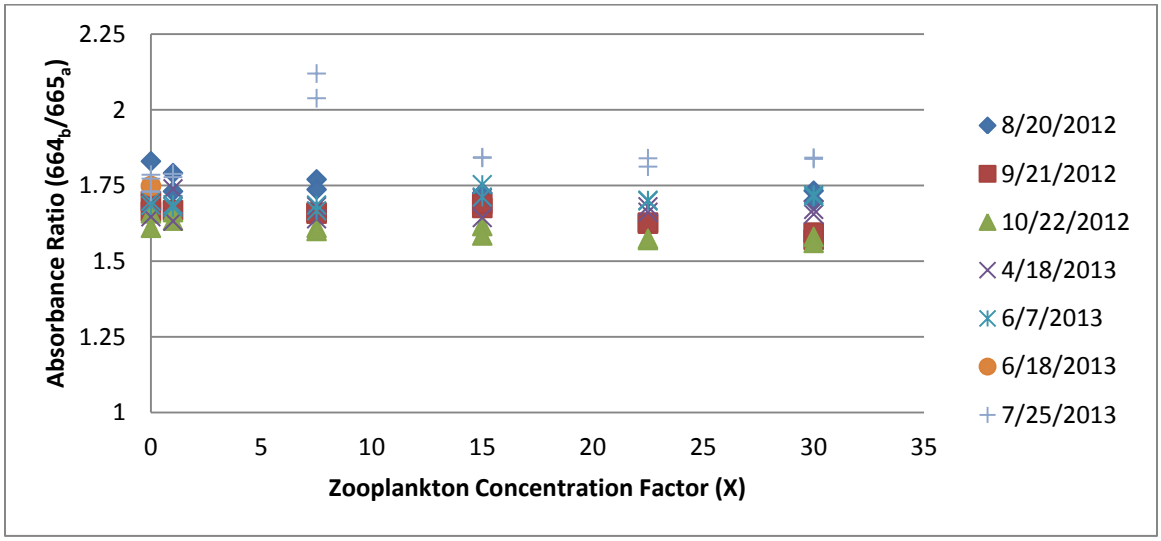

Figure 20. Absorbance ratios for final (post-incubation) light bottle microcosms using the concentrated grazers method.

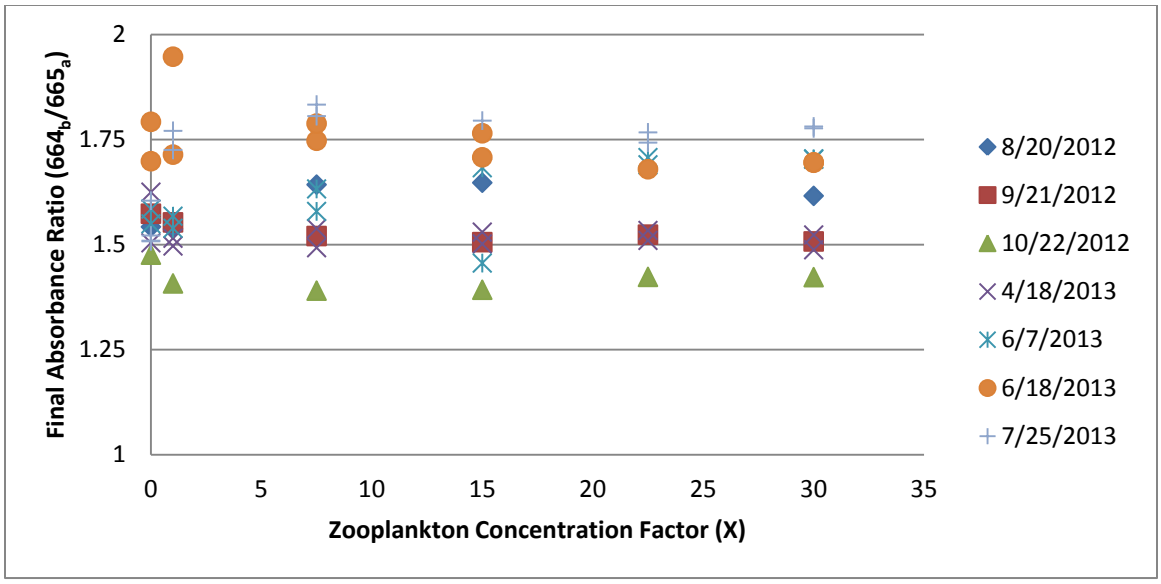

Figure 21. Absorbance ratios for final (post-incubation) dark bottle microcosms using the concentrated grazers method.

Grazing results. Studies were conducted using the grazer concentration method from August, 2012 through July, 2013. The chlorophyll *a* changes for each study date are plotted against zooplankton concentration factor and presented in Figures 19-25. Calculations were done using averaged chlorophyll *a* concentrations (see APPENDIX A. CHLOROPHYLL ANALYSIS for raw values) between samples, and a carbon to zooplankton biomass ratio of 0.45 (Hansen et al. 1997).

The slope of the linear regression fit line, k (d^{-1}), can be converted into a clearance coefficient, C ($m^3gC^{-1}d^{-1}$) using the carbon to zooplankton biomass ratio of 0.45 and the natural zooplankton density obtained from the 1X microcosms. The conversion is as follows:

$$C = \frac{k\ (d^{-1})}{Z\left(\frac{\mu g - biomass}{L}\right) * 10^{-6}\left(\frac{\mu g}{g}\right) * 0.45\left(\frac{gC}{g - biomass}\right) * 1000(\frac{L}{m^3})}$$

All samples exhibited minimal variation, which is discussed later. Only the August 20, 2012 trial exhibited anomalous data that were excluded from the regression, as shown in Figure 22. While the two preceding zooplankton density microcosms produced a decline in chlorophyll *a* production, the 30X microcosms exhibited an increase in chlorophyll *a* production. However, only the 30X microcosm data yielded reasonable grazing rate coefficients when algal productivity was considered in the analysis. Thus, the 7.5 and 15X data points were removed from the regression. This reason for exclusion is not justifiable, thus, in discussion of the modified concentrating grazers method, the August 20, 2012 results are omitted. It is worth restating that this was a preliminary experiment, and the results are relatively insignificant. This experiment served merely as a proof of concept.

The dark microcosms from August 20, 2012 exhibit no apparent pattern. The results from this experiment show that grazing was not a function of zooplankton density; however, with such poor R^2 values, the reliability of this dataset it questionable. However, this pattern set forth more interest in the dark microcosms for future studies.

The results from Figure 22 and a natural zooplankton density of 85.88-µg/L yielded clearance coefficients, C, of 0.564-$m^3gC^{-1}d^{-1}$ and 0.43-$m^3gC^{-1}d^{-1}$ for the light and dark microcosms of the August 20, 2012 test study, respectively. These values are near the typical range of 0.5-5.0- $m^3gC^{-1}d^{-1}$ for clearance coefficients (Chapra 1997); however, the significance of these results is minute due to the reasons previously explained.

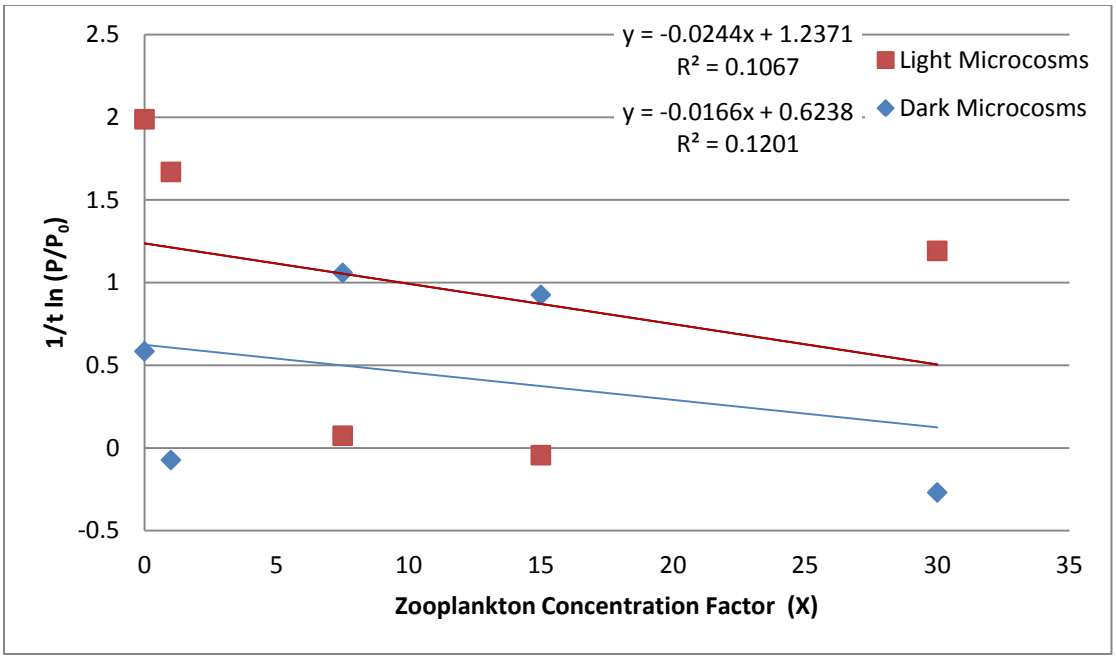

Figure 22. Fitted linear regression for the August 20, 2012 grazing study light and dark microcosms.

Figure 23 shows the September 21, 2012 study. With an R^2 of 0.9277, this was the most strongly linear result achieved. With a relatively high natural zooplankton concentration, the grazing effect is clearly visible, and increases with increasing zooplankton density. A natural zooplankton density of 53.53-µg/L yields a clearance coefficient, C, of 3.40-$m^3gC^{-1}d^{-1}$ for the light bottle microcosms and 0.934-$m^3gC^{-1}d^{-1}$ for dark bottle microcosms.

The dark microcosms in Figure 23 illustrate a pattern different than that shown in Figure 22. The dark September 21, 2012 microcosms illustrate a pattern similar to that of the light samples from the same date: a consistent increase of grazing activity as

zooplankton density increases. This pattern was expected; however, the other studies did not experience such favorable results.

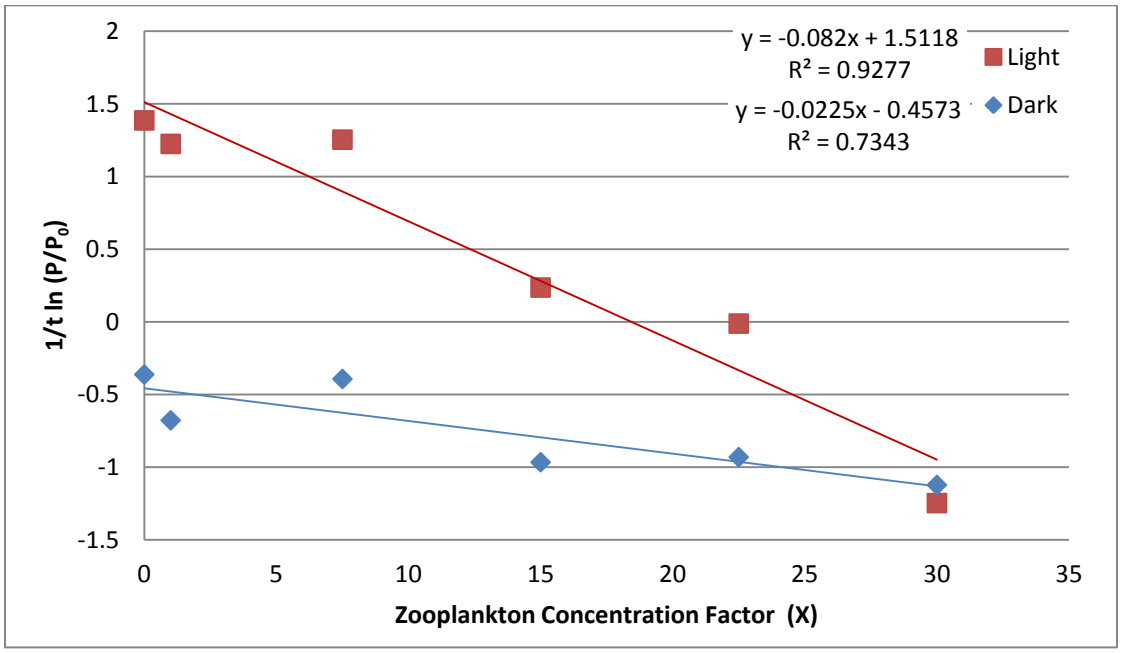

Figure 23. Fitted linear regression for the September 21, 2012 grazing study light and dark microcosms.

Figure 24 presents the results from the October 22, 2012 study, which showed a moderately strong linear correlation (R^2 of 0.762 for light bottle microcosms). The natural zooplankton concentration was 14.47-µg/L was typically low; however, chlorophyll *a* concentrations ranged from 4-11-µg/L, yielding clearance coefficients, C, of 2.27-$m^3gC^{-1}d^{-1}$ and 1.41-$m^3gC^{-1}d^{-1}$ for light and dark bottle microcosms, respectively.

Interestingly, the grazing impact appears to saturate at zooplankton densities of 15, 22.5, and 30X.

The saturation of grazing impact can also be seen through the dark microcosms in Figure 24. With an R^2 of 0.4727, the results are only moderately linear, mostly due to the plateau experienced in the 15-30X microcosms, similar to that of the light microcosms.

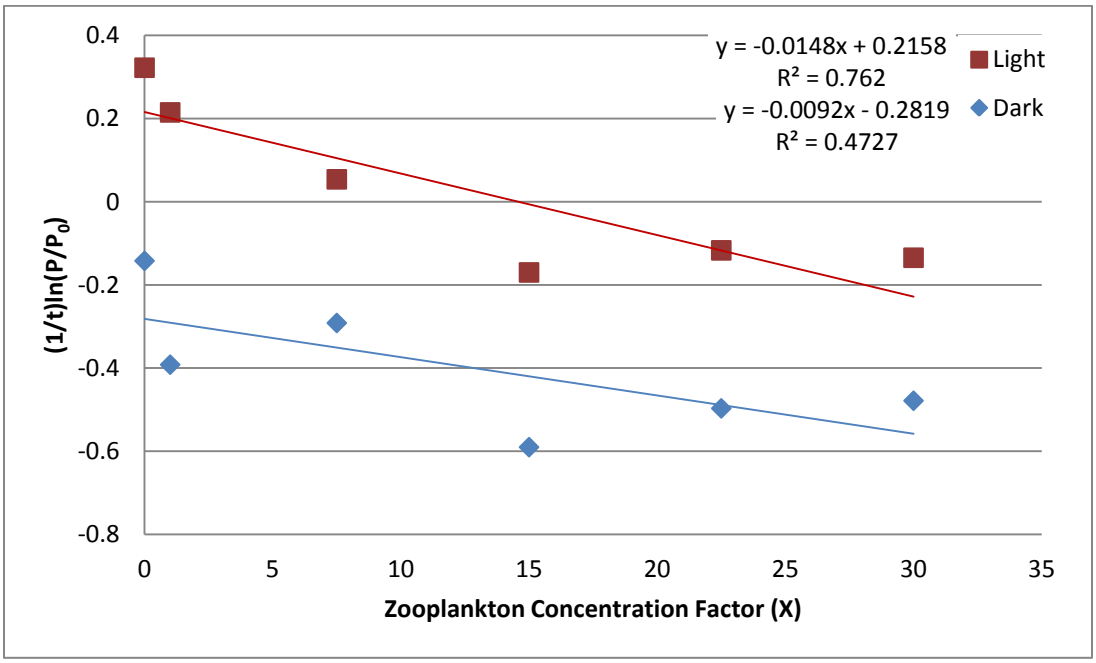

Figure 24. Fitted linear regression for the October 22, 2012 grazing study light and dark microcosms.

Figure 25 illustrates the results achieved from the April 18, 2013 grazing study. This study was completed in early spring, thus chlorophyll *a* concentrations were

moderate, ranging from 4-25-µg/L. Zooplankton counts showed a natural zooplankton density of 87.31-µg/L, yielding a clearance coefficient, C, of 0.295-$m^3gC^{-1}d^{-1}$ for light bottle microcosms and 0.015-$m^3gC^{-1}d^{-1}$ for dark bottle microcosms. As with the October 22, 2012 study, the grazing impact appears to plateau at the higher zooplankton densities.

The dark microcosms illustrated in Figure 25 exhibit no apparent pattern. The grazing does not increase with zooplankton density, but rather plateaus immediately. This poor linear relationship implies that grazing may not be solely reliant on zooplankton density.

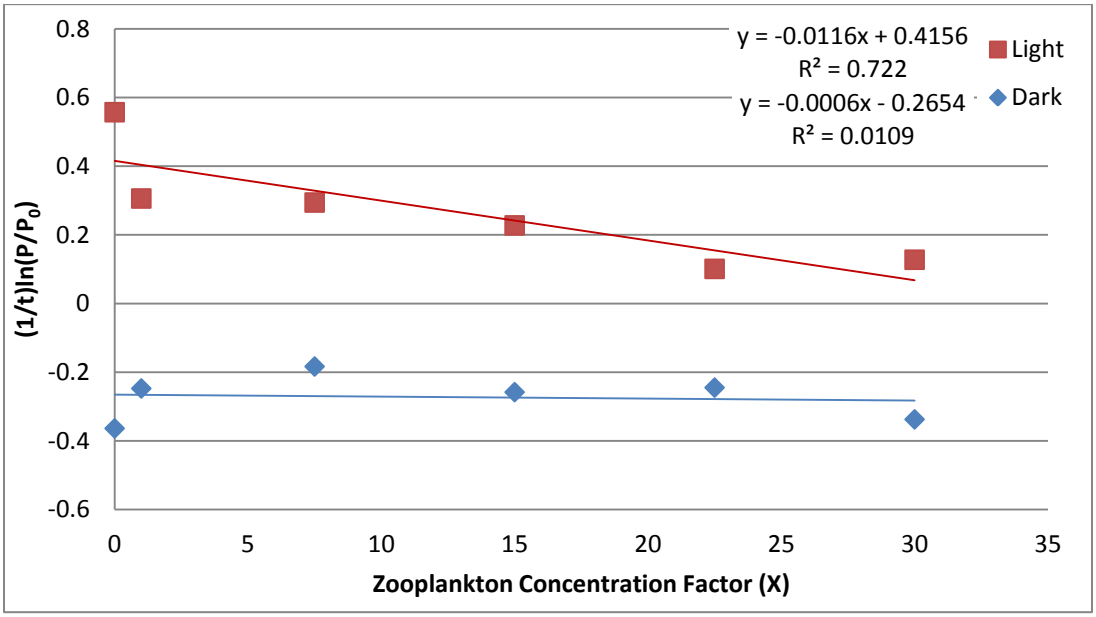

Figure 25. Fitted linear regression for the April 18, 2013 grazing study light and dark microcosms.

Figure 26 presents the results from the June 7, 2013 grazing study. A natural zooplankton density of 189.72-µg/L yielded clearance coefficients of 0.424-$m^3gC^{-1}d^{-1}$ and 0.006-$m^3gC^{-1}d^{-1}$ for light and dark bottle microcosms, respectively. The plateau of grazing impact experienced in the previous studies is seen in this study as well. Chlorophyll *a* concentrations ranged from 9-45-µg/L, which is moderately high for this section of the San Joaquin River.

The dark bottle microcosms did not experience an apparent grazing pattern. These results became typical for grazing experiments using this method; however, investigative capabilities are limited in grazing experiments, as only a few select factors (e.g. sunlight, temperature) can be controlled.

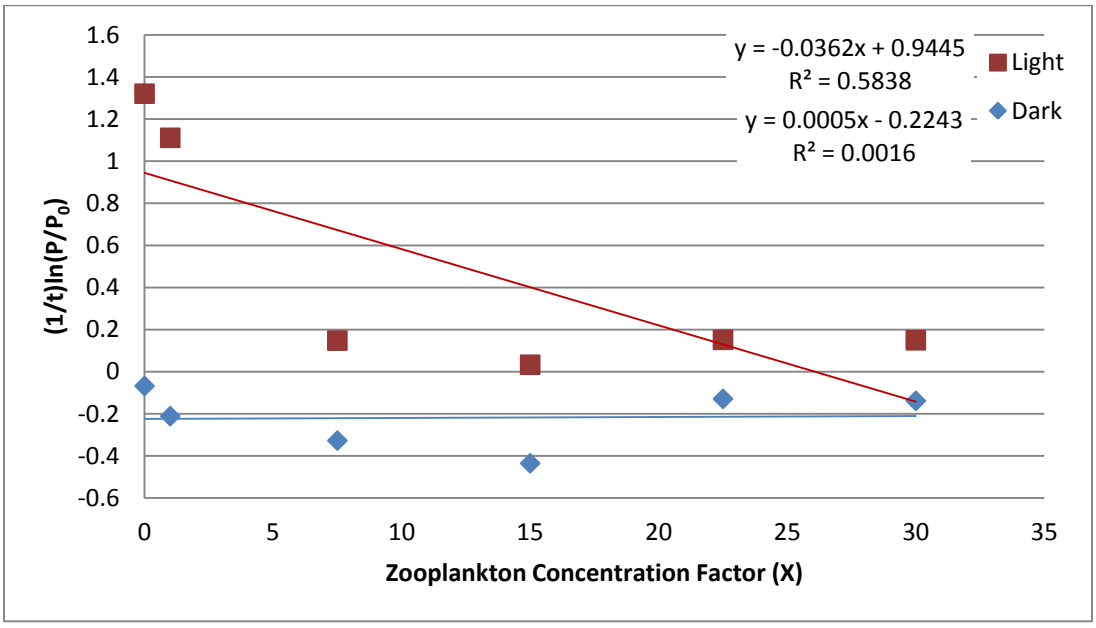

Figure 26. Fitted linear regression for the June 7, 2013 grazing study light and dark microcosms.

Figure 27 illustrates an evident grazing pattern, as the grazing impact increases with zooplankton density for the light microcosms. A natural zooplankton density of 131.31-µg/L yields clearance coefficients of 0.393-$m^3gC^{-1}d^{-1}$ and 0.030- $m^3gC^{-1}d^{-1}$ for light and dark bottle microcosms, respectively. There appears to be an anomaly with the 7.5X microcosm, which experienced a similar grazing effect to the 15X microcosm; however, the higher zooplankton densities are clearly distinguishable. Interestingly, the dark microcosms showed no such grazing pattern. With the linear regression analysis producing an R^2 of 0.182, there is no detectable grazing pattern, suggesting that the grazing impact in all samples is relatively equal. This contrasts with the results achieved in the light microcosms. Considering that chlorophyll *a* concentrations ranged from 5-44-µg/L, perhaps the zooplankton reached a feeding saturation, where they could no longer induce a greater grazing impact.

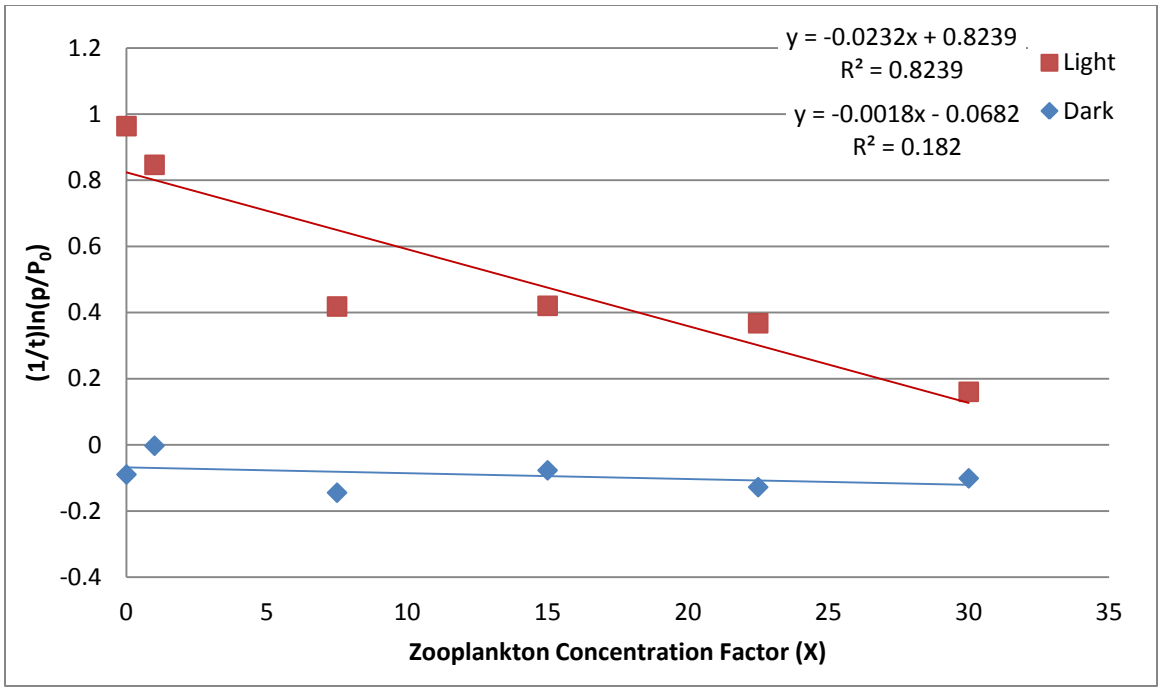

Figure 27. Fitted linear regression for the June 18, 2013 grazing study light and dark microcosms.

The results from the July 25, 2013 grazing study, as shown in Figure 28, exhibit a similar plateau pattern as many of the previous studies. A natural zooplankton density of 138.97-µg/L yielded clearance coefficients of 0.545-$m^3gC^{-1}d^{-1}$ and 0.154- $m^3gC^{-1}d^{-1}$ for light and dark bottle microcosms, respectively.

At the higher zooplankton densities, grazing appears to reach a maximum, which it maintains throughout the microcosms. Chlorophyll *a* concentrations ranged from 5-37-µg/L, which are moderate for this region. The dark microcosms have a moderately strong linear relationship, which suggests that grazing was increasing with zooplankton density.

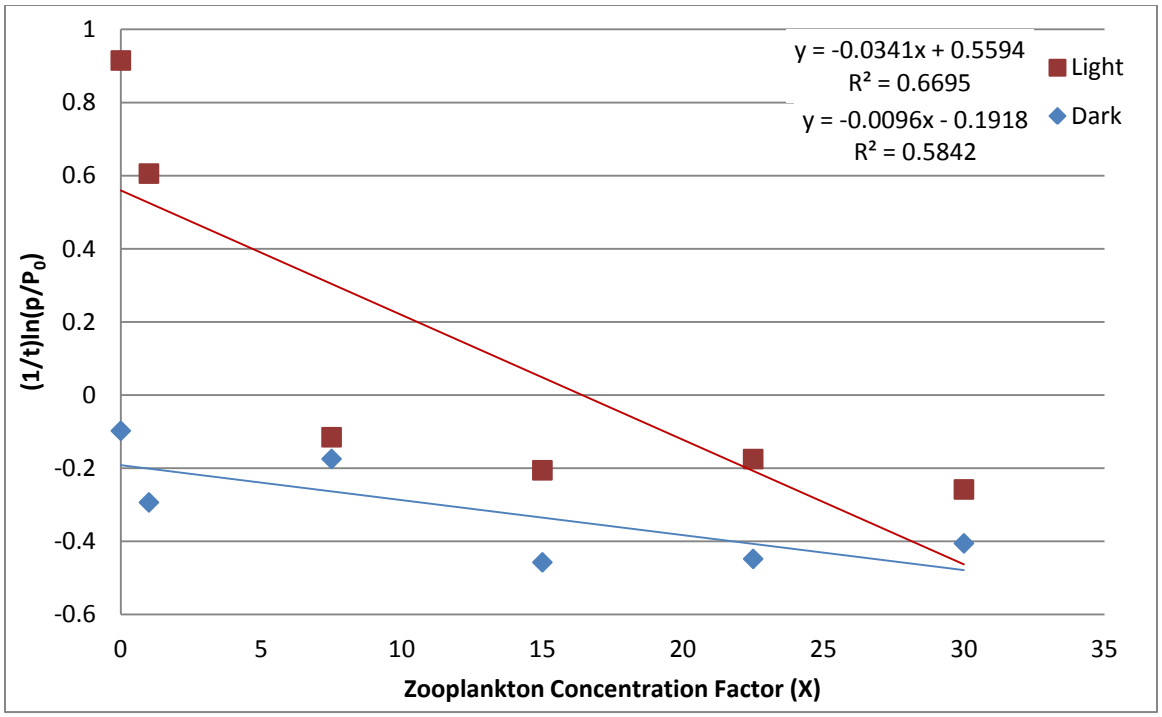

Figure 28. Fitted linear regression for the July 25, 2013 grazing study light and dark microcosms.

Table 4 presents the information relating to calculation of grazing rates, as well as the range of grazing rates calculated for the studies completed between 2012 and 2013.

Table 4. Zooplankton grazing rate coefficients measured for the grazer concentration method light and dark microcosms performed from 2012 to 2013.

Date	Initial Range of Chlorophyll *a* (µg/L)	Zooplankton (unconcentrated) (µg/L)	Light Algal Clearance Coefficient (C) ($m^3gC^{-1}d^{-1}$)	Dark Algal Clearance Coefficient (C) ($m^3gC^{-1}d^{-1}$)
August 20, 2012	4.24 - 13.27	85.88	0.564	0.430
September 21, 2012	8.32 – 14.23	53.53	3.404	0.934
October 22, 2012	4.90 – 10.20	14.47	2.273	1.413
April 18, 2013	4.68 – 21.12	87.31	0.295	0.015
June 7, 2013	10.61 – 38.48	189.72	0.424	0.006
June 18, 2013	6.73 – 36.63	131.31	0.393	0.030
July 25, 2013	5.08 – 36.82	138.97	0.545	0.154

The dynamics of zooplankton feeding at high zooplankton densities are relatively unknown, but the grazing experiments conducted from August 2012 through July 2013 suggest possible feeding saturations. Interestingly, this pattern was not seen in every experiment, suggesting that chlorophyll *a* concentrations have some importance to feeding dynamics. The San Joaquin River is a unique environment, so these dynamics will be further discussed later.

Nutrient Results

As previously stated, nutrient samples were analyzed for most of the grazing studies conducted between years 2012 and 2013. While nutrient limitations were never a

real concern considering the history of San Joaquin River nutrient loadings, these analyses were done as a precautionary measure. Table 5 shows the nutrient concentrations before and after the incubation periods (where applicable). For the studies using the grazer concentration method, 30X microcosm nutrient results are presented, as these are the microcosms where nutrient limitations would most likely be present. Significant differences in nutrients were not observed before and after the incubations periods. In all cases, addition of nutrients was deemed unnecessary, and thus, to simplify field procedures, was ignored. Nutrient changes before and after the incubation periods were minimal and likely associated with analysis errors.

Table 5. Nutrient concentrations before and after microcosm incubation by sample date. October, 2012 – July, 2013 nutrient values are from 30X zooplankton concentration factor microcosms.

Date	Nutrients Added (Y/N)	Nitrate Concentration (mg/L - Initial/Final)	Phosphate Concentration (mg/L - Initial/Final)
June 29, 2012	N	NA	NA
July 17, 2012	Y	NA/0.821	NA/0.199
July 31, 2012	N	NA	NA
August 13, 2012	N	NA	NA
August 20, 2012	N	NA	NA
September 21, 2012	N	NA	NA
October 22, 2012	N	NA/2.315	NA/0.348
April 18, 2013	N	2.963/3.022	0.334/0.388
June 7, 2013	N	1.560/1.459	0.312/0.350
June 18, 2013	N	1.986/1.947	0.475/0.408
July 25, 2013	N	1.589/1.421	0.6147/0.5332

Algal Productivity and Respiration

The zooplankton grazing microcosm studies completed between 2012 and 2013 also provided information about algal productivity and respiration rates. Figure 29 presents a general respiration rate achieved from all studies using the concentrating grazers method. The fitted first order decay curve yields a respiration rate, k_r, of approximately 0.3695-d^{-1} (R^2 of 0.7739).

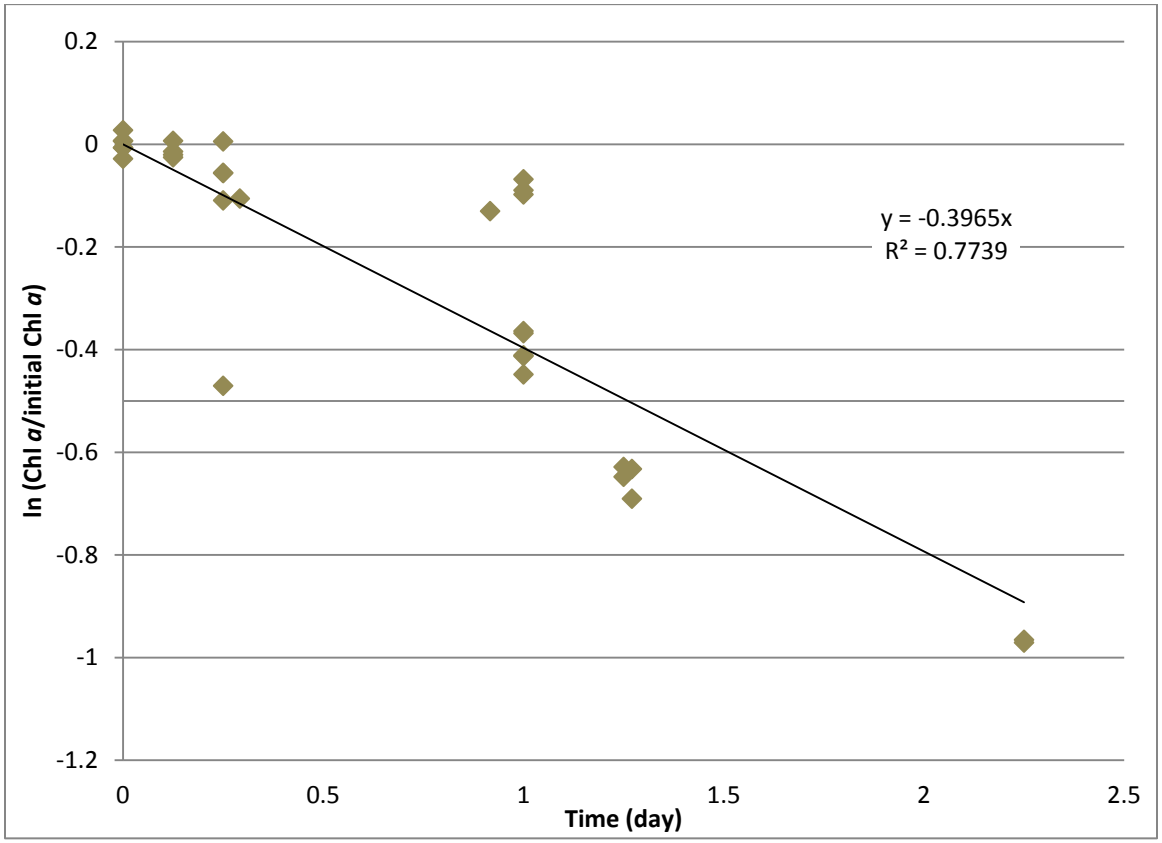

Figure 29. Algae respiration (slope) rates measured in dark bottles without zooplankton. Data from trials performed from August 20, 2012 to July 25, 2013.

4. Discussion

This chapter serves as a discussion of the results presented in Chapter 3. Each method is discussed for its strengths and weaknesses, and what it represents in terms of understanding the zooplankton-phytoplankton relationship in the San Joaquin River.

The Dilution Method

Application of the dilution method, as shown in Figures 10 and 12, was unsuccessful in the San Joaquin River. These experiments failed to produce measurable zooplankton grazing rates, for several reasons. Phytoplankton production in both trials was dominant as seen in Figures 9 and 11. Phytoplankton growth, as measured by chlorophyll changes pre and post incubation, during the June 29, 2012 study (Figure 9) ranged from 4-35-µg/L, with similar growth patterns occurring in the July 17, 2012 (Figure 11) study (ranged from 1-36-µg/L). Both studies contained significant zooplankton populations (14.99 and 200-µg/L on June 29 and July 17, 2012, respectively); however, grazing effects appeared to be small relative to the high phytoplankton growth rates. Since this method relies on the serial dilution of zooplankton populations, the effect of grazing on phytoplankton was further reduced. In addition, the changes in chlorophyll *a* in samples with and without zooplankton provide evidence that grazing was insignificant as compared to chlorophyll growth.

Grazing patterns obtained from the June 29 and July 17, 2012 study dates were similar to those of Tijdens et al. (2008). Several factors that induce poor results could be associated with variations in phytoplankton populations between microcosms, variations in zooplankton populations between microcosms, and high algal productivity rates (as stated previously). These factors are uncontrollable for in-situ experiments, as zooplankton and phytoplankton populations were captured from natural environments, rather than laboratory prepared. The species and quantity of zooplankton within the microcosms are unknown until after experimentation; whether the zooplankton within the microcosms will feed on the available phytoplankton is unknown. This method might be better suited for laboratory experiments where specific zooplankton species (e.g. copepods) can be placed in microcosms to determine feeding rates. For example, significant research has been done to determine the feeding rates of copepods, including investigations by Gauld (1951) and Roman et al. (1988). Specific species grazing is different than community grazing, which yield estimates of overall grazing rates. In addition, grazing patterns are dependent upon several factors (e.g. time of day, temperature) that vary for different species; these factors can be better controlled in a laboratory setting.

The Food-Removal Method

The food-removal method results were shown in Figures 13 and 14. Figures 13 and 14 suggest that phytoplankton mortality was largely due to respiration, as shown by the similar linear regression line slopes of microcosms with and without grazers. These

studies also failed to produce measurable grazing rates. Zooplankton analysis on August 13, 2012 revealed a zooplankton density of 54.78-µg/L; however, the grazing effect by these zooplankton was undetectable. This behavior could not be due to phytoplankton production; since all microcosms were incubated in darkness.

The food-removal method studies did provide insight into the effect of mixing on phytoplankton behavior within the microcosms. A subset of microcosms was mixed to determine the influence on mixing on grazing and respiration. From Figures 13 and 14, unmixed samples experienced faster phytoplankton mortality rates (steeper slopes) than mixed microcosms (Figure 13: -0.4744 unmixed compared to -0.3922 mixed; Figure 14: -0.6645 unmixed compared to -0.578 mixed). This mortality could be associated with respiration, grazing, or viral losses. Whether settling had an influence on grazing is unknown, as grazing was immeasurable during these studies, as shown by the similar chlorophyll *a* slopes for microcosms with and without zooplankton. However, this observation does suggest that phytoplankton health may be dependent upon mixing within the microcosm. Studies using field incubation techniques are unable to produce significant mixing within the microcosms, which could contribute to phytoplankton mortality. Since laboratory incubation is not always feasible (e.g. time constraints, light conditions), understanding that consistently mixing microcosms possibly promotes phytoplankton health should be noted and investigated further.

The Modified Grazer Concentration Method

The grazer concentration method was utilized because of its ability to produce measureable grazing rates, when other methods (e.g. dilution, food-removal) failed. Capriulo and Carpenter (1980) first used this method; however, our modifications allowed this method to be in-situ. By capturing zooplankton with a zooplankton trap, laboratory preparation of zooplankton was unnecessary, and field microcosm preparation was enable. Figures 22-28 present the results of grazing trials performed with zooplankton concentrations up to 30 times their natural densities. Measurable rates were obtained because zooplankton concentrations were amplified to a level where the grazing effect by zooplankton populations was noticeable. Clearance rates (Table 4: light: 0.295-3.404-$m^3gC^{-1}d^{-1}$, dark: 0.006-1.413-$m^3gC^{-1}d^{-1}$) were near or within the typical range proposed by Chapra (1997), suggesting that this is a viable method for estimating zooplankton grazing impacts. Light bottled microcosms exhibited moderate to good linear fits (Figures 22-28: R^2: 0.5838-0.9277); however, a plateau effect was observed in high zooplankton density microcosms (22.5, 30X). There are a few possible sources for this effect, which are discussed later in this chapter.

Discussion of preparation and analysis methods. This section discusses the preparation and analysis techniques used for the grazing experiments and the validity of these methods.

Chlorophyll a concentrations. The food-removal and grazer concentration method analyses presented in Chapter 3 used averaged chlorophyll *a* concentrations to represent a subset of samples. Averages were solely used in the modified concentrated

grazer studies because of the consistency observed in chlorophyll analysis between similar samples (see APPENDIX A. CHLOROPHYLL ANALYSIS). As shown in Figure 11, the algal growth during the incubation period for similar samples is typically 0-7-$\mu gL^{-1}d^{-1}$, suggesting similar phytoplankton populations within similar microcosms. This is also illustrated in Figures 19-21, showing similar absorbance ratios for similar samples. The maximum standard deviations for similar samples are 0.052, 0.079, and 0.165 for initial, final light, and final dark microcosms, respectively. These figures show accuracy in preparation, analysis and experimental methods.

Duplicate samples were typically prepared for each sample subset; however, single samples were used for initial chlorophyll samples in 2013 because half of each sample was designated for zooplankton analysis. Duplicate samples were analyzed for all final chlorophyll concentrations in all the grazing trials.

The consistency and accuracy in the chlorophyll analysis methods used is sufficient to dismiss this as a potential factor in microcosm grazing rate variation. The patterns observed in the 2012-2013 grazing studies are likely due to other factors present within the microcosms during the incubation period; however, variations in chlorophyll analysis are insignificant and do not noticeably effect grazing rates.

Zooplankton analysis. Zooplankton analyses were conducted for every experimental study; however, the 2013 modified concentrated grazers studies were given increased interest in zooplankton activity within the microcosms during the incubation period, necessitating the analysis of zooplankton before and after incubation periods. Figures 15-18 illustrate the total zooplankton densities within a representative microcosm

for the concentrated grazers method as a function of the designed zooplankton concentration factor (X). All samples contained initial zooplankton concentrations near their designed concentrations (see Table 3 for R^2 values), illustrating that the preparation technique for these samples is sufficient. In addition, Figures 15-18 illustrate accuracy in the zooplankton measurement method.

Contrary to the initial zooplankton densities measured within the microcosms, the final zooplankton densities experienced decreases. On the April 18, June 18, and July 25, 2013 grazing trials, the high density zooplankton microcosms experienced significant decreases in zooplankton density. A comparison of grazing coefficients calculated using both the theoretical and final zooplankton concentration factors is shown in Table 6.

Table 6. Comparison of grazing rates, clearance rates, and R^2 values calculated from theoretical and final zooplankton concentration factors for light and dark microcosms using the grazer concentration method.

Study Date	Initial Grazing Rate (d^{-1})	Initial Clearance Rate ($m^3gC^{-1}d^{-1}$)	Initial R^2	Final Grazing Rate (d^{-1})	Final Clearance Rate ($m^3gC^{-1}d^{-1}$)	Final R^2
04/18/13 Light	0.0116	0.295	0.7220	0.0190	0.484	0.6243
06/07/13 Light	0.0362	0.424	0.5838	0.0831	0.973	0.9646
06/18/13 Light	0.0232	0.393	0.8239	0.0083	0.140	0.1110
07/25/13 Light	0.0341	0.545	0.6695	0.0806	1.289	0.8253
04/18/13 Dark	0.0006	0.015	0.0109	0.0021	0.053	0.0465
06/07/13 Dark	0.0005	0.006	0.0016	0.0128	0.150	0.3868
06/18/13 Dark	0.0018	0.030	0.1820	0.0004	0.007	0.0087
07/25/13 Dark	0.0096	0.154	0.5842	0.0217	0.347	0.6604

From Table 6, using the final zooplankton concentration factors observed in the microcosms alters the clearance rates dramatically. There is not a consistent pattern of change in the clearance rates due to using the theoretical or final zooplankton concentration factors; however, noting these changes is of interest. The final zooplankton densities do not follow the theoretical concentration lines (see Figures 15-18), implying that zooplankton densities were fluctuating within the microcosms. Clearance rates for light bottled microcosms typically increased (with the exception of the June 18, 2013 trials). In addition, there were few small-bodied grazers present during final zooplankton analysis, suggesting that the large bodied grazers contributed the majority of grazing in

the microcosms. The contrast between initial and final zooplankton concentration factors could significantly alter the grazing rates obtained by the grazer concentration method. It was expected that zooplankton densities were not constant in the microcosms throughout the incubation periods; however, using the initial zooplankton densities appears to underestimate the clearance rates significantly. A reasonable assumption is that the actual clearance rates are situated between the two rates presented in Table 6.

Nutrient limitations. Nutrient limiting conditions in the microcosms were not expected during the grazing studies performed from 2012-2013 due to high background concentrations in the San Joaquin River. However, nutrient analysis was performed on most occasions to verify this assumption. Table 5 presents the nitrate, phosphate, and silica species analysis results for the various grazing studies. As seen in Table 5, nutrient concentrations before and after the incubation periods were similar for all microcosms, suggesting nutrients were consistently available for aquatic organisms within the microcosms.

Several grazing trials from 2012-2013, most notably those is Figures 24-26 and 28, experienced plateaus in grazing at high zooplankton concentration factors. While nutrients have a significant effect on microorganism activity within a water body, the observed plateaus are not likely attributed to nutrient uptake limitations.

Discussion of zooplankton populations. Zooplankton densities were measured before and after incubation periods for each microcosm concentration (see Figures 15-18). The initial zooplankton densities were near their designed values; however, declines in densities were experienced in the high density microcosms (15, 22.5, and 30X).

Reasoning for this is unknown; however, several factors could contribute to this occurrence. Nutrient limitations were not present (as previously discussed). In addition, food limitations were not present, as the higher density microcosm contained the highest amounts of chlorophyll *a*, and contained significant amounts post-incubation (see APPENDIX A. CHLOROPHYLL ANALYSIS).

One factor that could have caused zooplankton mortality is bacterial growth. The microcosms were sealed, suggesting organism waste products would be kept within the microcosm. In a natural setting, waste products would be removed from an organism's location, either by settling or water flow, thus bacteria feeding on these waste products would not be located near the waste source. Enveloping many members of the microorganism food chain in a 1-L microcosm can produce unexpected stresses, such as bacterial growth, that could produce unforeseen zooplankton mortality.

Another contributing factor to zooplankton mortality is the spatial limitations present within the microcosm. Subjecting any organism to a confined space smaller than their natural habitat will induce stress. It is a possibility that containing high densities of zooplankton in a space-limited microcosm for 24 hour durations caused unexpected mortalities.

Issues with all microcosm experiments are inherent to isolating a water sample and are often unavoidable. However, this is currently the most feasible method of measuring zooplankton grazing.

Discussion of phytoplankton populations. Similar to zooplankton populations within the microcosms, phytoplankton populations were also placed in a stressful

environment. To test whether phytoplankton populations remain in good physiological health through the experimental process, ratios of absorbance wavelengths 664-nm (before acidification) and 665-nm (after acidification) were presented in Figures 19-21. Figure 19 shows these ratios before the incubation period, and Figures 20 and 21 present these ratios after the incubation period for the light and dark microcosms, respectively.

Initially, all trials contained healthy phytoplankton populations for all microcosms, as absorbance ratios ranged from 1.5-2.0 for most samples. A healthy algal community will typically exhibit a ratio of 1.7 and unhealthy communities will yield absorbance ratios of 1.0, indicating all chlorophyll *a* has decomposed to pheophytin *a*. Figure 19 ensures that the sample preparation process for the grazer concentration method is not harming phytoplankton.

Figures 20 and 21 also illustrate that the phytoplankton populations maintained their health throughout the incubation period. Absorbance ratios for the light microcosms (Figure 20) were generally higher than those of the dark microcosms (Figure 21). Since phytoplankton require sunlight to live, this was expected. Figure 21 also illustrates that the zooplankton were provided with prey throughout the incubation period, as the phytoplankton populations were able to endure a prolonged period of darkness.

Discussion of grazing rates. Figures 22-28 illustrate the zooplankton grazing patterns observed within the grazer concentration method microcosms. Generally, this method produced measureable grazing rates, with moderate to good linear fits for the light bottle microcosms, as stated previously. Clearance rates were typically within the range proposed by Chapra (1997) of 0.5-5.0-$m^3gC^{-1}d^{-1}$, suggesting this is a viable method

for estimating zooplankton grazing impacts in this study location. However, Figures 24-28 exhibit an unexpected pattern associated with the high zooplankton density (15, 22.5, and 30X) microcosms, in which the grazing effect appears to saturate. Referring to Figures 15-18, this pattern could be induced by the mortality of zooplankton in the high density microcosms. As previously discussed, zooplankton populations in the high density microcosms often decreased during the incubation periods. While the cause of this mortality is unknown, the decrease in zooplankton population may partially explain the decrease in grazing.

Unexpected results were obtained from the dark bottle microcosms; these trials were unable to produce consistent grazing patterns. Table 4 shows the clearance rates obtained from the dark bottle microcosms, which were typically within the range proposed by Chapra (1997) (range of C: 0.006-1.413-$m^3gC^{-1}d^{-1}$); however these experiments produced poor linear fits (R^2 range of 0.0016-0.7343; average R^2 of 0.3008). Roman et al. (1988) observed that copepods consume 90% of their daily food intake during dark hours, likely to avoid visually feeding predators. However, the results obtained from dark microcosms suggest lower grazing rates in darkness, compared to grazing rates in light microcosms. Causes for this observation are unknown, but could be related to zooplankton species within the microcosms. Zooplankton counts revealed a variety of different zooplankton species within the microcosms (see APPENDIX B. ZOOPLANKTON ANALYSIS); all zooplankton species feed differently and in different conditions (e.g. darkness, daytime, different temperatures, different algal levels). Current methods do not allow for selection of zooplankton species within the microcosm, thus the amounts of each type of zooplankton within microcosms are not identical.

Clearance rates are known to depend on many factors that include algae saturation (Mullin et al. 1975; Fenchel 1982), lower feeding thresholds (Frost 1975; Kiorboe et al. 1985; Jeong et al. 2007), zooplankton species and size selectivity (Hansen et al. 1994; Hansen et al. 1997; Roman et al. 1988), acclimation to ambient food and the influence of algal production rates (Sanderson et al. 2012). The modified grazer concentration method studies performed from 2012-2013 showed a strong grazing dependence on phytoplankton production as indicated by higher grazing rates measured in light than dark microcosms.

Additionally, clearance rates are dependent on phytoplankton levels, specifically, when saturation levels occur. Bowie et al. (1985) suggest that half-saturation values for chlorophyll *a* range from 5.0-15-µg/L. Chlorophyll *a* concentrations range from 5-100-µg/L in the DWSC (Lee and Lee 2000). Fenchel (1982) suggested that clearance rates decrease as phytoplankton approaches saturation levels. In the grazer concentration method studies, phytoplankton populations increased with zooplankton concentration up to 45-µg/L (see APPENDIX A. CHLOROPHYLL ANALYSIS); this could explain the grazing saturation in high zooplankton density microcosms (15, 22.5, and 30X). The most linear results were achieved during the September 21, 2012 study (R^2 of 0.7343), in which phytoplankton populations were low (<15-µg/L). In addition, this study only used a 7 hour incubation period, indicating that a 24 hour dark incubation period might dull the grazing effect. Further investigation into this occurrence is needed.

In addition to differences between light and dark microcosms, seasonal differences in grazing patterns were observed. The 2012 studies were conducted in late summer to fall (August-October, 2012) and produced higher grazing rates than the 2013

studies (conducted in spring to summer, April-July, 2013). Phytoplankton populations were typically lower in the 2012 studies (less hours of sunlight, colder temperatures); however, grazing was not expected to be significantly higher in these trials than in the 2013 trials. The ranges of clearance rates in 2012 and 2013 were 0.564-3.607-$m^3gC^{-1}d^{-1}$ and 0.295-0.545-$m^3gC^{-1}d^{-1}$. A possible cause of this observed pattern is that chlorophyll levels were below saturation levels in 2012, thus zooplankton grazing was not limited, as Fenchel (1982) suggests. Additional grazing trials are needed to confirm if this behavior is reproducible or a random occurrence.

While several mechanisms may explain the grazing patterns observed in the grazer concentration studies, it is unclear whether a single cause can be identified. The observed behavior may be the result of a combination of factors, but the determination of this is outside of the scope of this work. However, the observation of such zooplankton-phytoplankton interactions may be an important area for future research.

5. Conclusions

Measuring zooplankton grazing has rendered many research efforts as scientists try to understand the underlying microorganism dynamics of various water bodies. Zooplankton and phytoplankton exist in a balance in which the success of one organism is largely dependent on the success of the other. Research completed between 2012 and 2013 in the San Joaquin River attempted to quantify zooplankton grazing rates to better understand this relationship.

The dilution method (Landry and Hassett 1982) produced immeasurable grazing rates. This was due to serial dilutions of zooplankton populations, which decreased the measurable grazing effects. Because our study location in the San Joaquin River was highly productive, zooplankton grazing was typically masked by phytoplankton growth. A highly productive environment for algae and serial dilutions of zooplankton within the microcosms produced results indicating that the dilution method was unsuitable for this study location.

The food-removal method (Gauld 1951) experiments uncovered many factors about zooplankton-phytoplankton interaction in the San Joaquin River. Firstly, zooplankton populations are highly variable. Because the San Joaquin River is subject to different flow, vegetative, and bathymetric conditions (anthropogenic or naturally caused) at different stretches, the organisms within the river are inconsistent. The food-

removal method also revealed that there is a possible dependence of zooplankton grazing upon phytoplankton production, as all trials using this method were conducted in dark environments and grazing was immeasurable.

While the dilution and food-removal methods failed to produce measurable grazing rates, a modified concentrated grazer method succeeded. By concentrating zooplankton up to 30 times their natural densities, grazing and clearance rates were measureable, and were typically within the range of literature values (Chapra 1997). In addition to producing measurable grazing rates, this method also revealed relationships between zooplankton and phytoplankton in the San Joaquin River. Zooplankton were observed to exhibit predictable grazing patterns in light conditions, while dark conditions resulted in poorly linearly correlated grazing patterns. This observation was contrary to literature suggestions (e.g. Roman et al. 1988); however, it may suggest that other undiscovered factors are important to grazing rates in the San Joaquin River. Development of a water quality model with zooplankton-phytoplankton interactions could help to elucidate and prioritize important mechanisms and guide future zooplankton grazing investigations in this region.

The grazing trials conducted between 2012 and 2013 also present several obstacles for future researchers in this area. One obstacle is the inability of closed microcosms to present accurate grazing measurements. Experiments revealed decreases in zooplankton populations between during microcosm incubation, suggesting that zooplankton mortality could be significant. While the source of this mortality is unknown, future researchers could reduce the stress (e.g. bacterial growth, space limitations, etc.) placed on organisms within their microcosms by reducing the

concentration factor. In addition, much of the previous research in the area of zooplankton grazing measurement has presented results in units partial to its respective researchers (Hansen et al. 1997). This approach leads to results that are often difficult to compare directly. One of the goals of this research was to present results such that comparison is simple, or already complete. Researchers present either grazing rates (k [d^{-1}]) or clearance rates (C [$m^3gC^{-1}d^{-1}$]), but rarely both. Without the reporting of zooplankton concentrations (often the case), conversion between the two rates not possible. Future research in this area should strive to present results in a manner which facilitates conversion between grazing and clearance rates.

This research revealed many possible factors existing in the relationship between phytoplankton and zooplankton within the San Joaquin River; however, many of the factors in this relationship remain unknown. Future microcosm grazing research should incorporate possible factors (e.g. light/dark dependent grazing, bacterial growth within microcosms, settling within microcosms) to further improve our understanding of their significance. For example, possible anomalous zooplankton grazing under light and dark microcosms offer new opportunities for future research in the San Joaquin River. However, the implications of these grazing rates on algal populations and dissolved oxygen deficits in the San Joaquin River should first be evaluated with water quality model simulations to assess the importance of incorporating zooplankton-phytoplankton interactions in the tools used for resource management.

References

APHA (2005). *Standard Methods of the Examination of Water and Wastewater*, American Public Health Association, Washington, DC.

Balcer, M. D., Korda, N. L., and Dodson, S. I. (1984). *Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species*, University of Wisconsin Press, Madison.

Bernardi, R. d., and Giussani, G. (1990). "Are blue-green algae a suitable food for zooplankton? An overview." *Hydrobiologia*, 200(201), 29-41.

Bowie, G. L., Mills, W. B., Porcella, D. B., Campbell, C. L., Pagenkopf, J. R., Rupp, G. L., Johnson, K. M., Chan, P. W. H., and Gherini, S. A. (1985). "Rates, constants, and kinetics formulations in surface water quality modeling." U. S. E. P. Agency, ed., Environmental Research Laboratory, Athens, GA.

Capriulo, G. M., and Carpenter, E. J. (1980). "Grazing by 35 to 202 μm micro-zooplankton in Long Island Sound." *Marine Biology*, 56, 319-326.

Carlson, R. M. (1978). "Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide." *Anal. Chem.*, 50, 1528-1531.

Carlson, R. M. (1986). "Continuous flow reduction of nitrate to ammonia with granular zinc." *Anal. Chem.*, 58, 1590-1591.

Carlson, R. M., Cabrera, R. I., Paul, L., Quick, J., and Evans, R. Y. (1990). "Rapid direct determination of ammonium and nitrate in soil and plant tissue extracts." *Communications in Soil Science and Plant Analysis*, 21(13-16), 1519-1529.

Carrick, H. J., Fahnenstiel, G. L., and Taylor, W. D. (1992). "Growth and production of planktonic protozoa in Lake-Michigan - Insitu versus invitro comparisons and importance to food web dynamics." *Limnology and Oceanography*, 37(6), 1221-1235.

Chapra, S. C. (1997). *Surface Water-Quality Modeling*, McGraw-Hill, New York.

Chen, B., Landry, M. R., Huang, B., and Liu, H. (2012). "Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean?" *Limnology and Oceanography*, 57(0024-3590), 519-526.

Chengalath, R., Fernando, C. H., and George, M. G. (1971). "Planktonic rotifera of Ontario." *University of Waterloo Biology Series*, 2.

Cloern, J. E., and Jassby, A. D. (2010). "Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems." *Estuaries and Coasts*, 33, 230-241.

Cyr, H., and Pace, M. L. (1992). "Grazing zooplankton and its relationship to community structure." *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 1455-1465.

Desvilettes, C., Bourdier, G., Amblard, C., and Barth, B. (1997). "Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae." *Freshwater Biology*, 38, 629-637.

Dodds, W. K., and Oakes, R. M. (2008). "Headwater influences on downstream water quality." *Environmental Management*, 41, 367-377.

Dzialowski, A. R., Wang, S.-H., Lim, N.-C., Spotts, W. W., and Huggins, D. G. (2005). "Nutrient limitation of phytoplankton growth in central plains reservoirs, USA." *Journal of Plankton Research*, 27(6), 587-595.

E.P.A (2003). "Standard Operating Procedure for Zooplankton Analysis, LG403. In: Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes, Chapter 4. ."

Eloire, D., Somerfield, P. J., Conway, D. V. P., Halsband-Lenk, C., Harris, R., and Bonnet, D. (2010). "Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling." *Journal of Plankton Research*, 32(5), 657-679.

Enright, J. T. (1969). "Zooplankton grazing rates estimated under field conditions." *Ecology*, 50(6), 1070-1075.

Evans, C., Archer, S. D., Jacquet, S., and Wilson, W. H. (2003). "Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a Micromonas spp. population." *Aquatic Microbial Ecology*, 30, 207-219.

Fenchel, T. (1982). "Ecology of heterotrophic microflagellates. 2. Bioenergetics and growth." *Marine Ecology Progress Series*, 8, 225-231.

Figueiredo, G. M., Montagnes, D. J., and Nash, R. D. M. (2009). "The importance of protozooplankton as prey for copepods in the coastal areas of the central Irish Sea." *Hydrobiologia*, 628, 227-239.

Fileman, E., Smith, T., and Harris, R. (2007). "Grazing by Calanus helgolandicus and Para-Pseudocalanus spp. on phytoplankton and protozooplankton during the spring bloom in the Celtic Sea." *Journal of Experimental Marine Biology and Ecology*, 348.

Franks, P. J. S. (2002). "NPZ models of plankton dynamics: Their construction, coupling to physics, and application." *Journal of Oceanography*, 58(2), 379-387.

Frost, B. W. (1972). "Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*." *Limnology and Oceanography*, 17, 805-815.

Frost, B. W. (1975). "A threshold feeding behavior in *Calanus pacificus*." *Limnology and Oceanography*, 20, 263-266.

Gameiro, C., and Brotas, V. (2010). "Patterns of phytoplankton variability in the Tagus Estuary (Portugal)." *Estuaries and Coasts*, 33, 311-323.

Gauld, D. T. (1951). "The grazing rate of planktonic copepods." *Journal of the Marine Biological Association of the United Kingdom*, 29(3), 695-706.

Gowen, R. J., McCullough, G., Dickey-Collas, M., and Kleppel, G. S. (1998). "Copepod abundance in the western Irish Sea: relationship to physical regime, phytoplankton production and standing stock." *Journal of Plankton Research*, 20(2), 315-330.

Gowen, R. J., McCullough, G., Kleppel, G. S., Houchin, L., and Elliott, P. (1999). "Are copepods important grazers of the spring phytoplankton bloom in the western Irish Sea?" *Journal of Plankton Research*, 21(3), 465-483.

Gowen, R. J., Tett, P., Kennington, K., Mills, D. K., Shannon, T. M., Stewart, B. M., Greenwood, N., Flanagan, C., Devlin, M., and Wither, A. (2008). "The Irish Sea: Is it eutrophic?" *Estuarine, Coastal and Shelf Science*, 76, 239-254.

Greenwood, N., Forster, R. M., Creach, V., Painting, S. J., Dennis, A., Cutchey, S. J., Silva, T., Sivyer, D. B., and Jickells, T. (2012). "Seasonal and interannual variation of the phytoplankton and copepod dynamics in Liverpool Bay." *Ocean Dynamics*, 62, 307-320.

Haney, J. F. (1971). "An in situ method for the measurement of zooplanton grazing rates." *Limnology and Oceanography*, 16, 970-976.

Hansen, B., Bjornsen, P. K., and Hansen, P. J. (1994). "The size ratio between planktonic predators and their prey." *Limnology and Oceanography*, 39(2), 395-403.

Hansen, P. J., Bjornsen, P. K., and Hansen, B. W. (1997). "Zooplankton grazing and growth: Scaling within the 2-2,000-µm body size range." *Limnology and Oceanography*, 42(4), 687-704.

Kiorboe, T., Mohlenberg, F., and Riisgard, H. U. (1985). "In situ feeding rates of planktonic copepods: A comparison of four methods." *J. Exp. Mar. Biol. Ecol.*, 88, 67-81.

Kobayashi, T., and Church, A. G. (2003). "Role of nutrients and zooplankton on phytoplankton growth in a temperate reservoir in New South Wales, Australia." *Marine and Freshwater Research*, 54, 609-618.

Landry, M. R., and Hassett, R. P. (1982). "Estimating the grazing impact of marine micro-zooplankton." *Marine Biology*, 67, 283-288.

Landry, M. R., Kirshtein, J., and Constantinou, J. (1995). "A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific." *Marine Ecology Progress Series*, 120, 53-63.

Lee, G. F., and Lee, A. J. (2000). "Synopsis of issues in developing the San Joaquin River Deep Water Ship Channel DO TMDL."

Lehman, P. W., Sevier, J., Giulianotti, J., and Johnson, M. (2004). "Sources of oxygen demand in the lower San Joaquin River, California." *Estuaries*, 27(3), 405-418.

Litton, G. M., Brunell, M., Monroe, J. C., and Quinn, N. W. T. (2008). "Linking the San Joaquin River to the Stockton Deep Water Ship Channel, Task 8." *San Joaquin River Up-Stream DO TMDL Project, ERP-02D-P63*, University of the Pacific, 164.

Liu, Y., Evans, M. A., and Scavia, D. (2010). "Gulf of Mexico Hypoxia: Exploring increasing sensitivity to nitrogen loads." *Environmental Science and Technology*, 44, 5836-5841.

Lodi, S., Vieira, L. C. G., Velho, L. F. M., Bonecker, C. C., Carvalho, P. d., and Bini, L. M. (2011). "Zooplankton community metrics as indicators of eutrophication in urban lakes." *Brazilian Journal of Nature Conservation*, 9(1), 1-6.

M.Redden, A., Sanderson, B. G., and Rissik, D. (2002). "Extending the analysis of the dilution method to obtain the phytoplankton concentration at which microzooplankton grazing becomes saturated." *Marine Ecology Progress Series*, 226, 27-33.

Meybeck, M., and Moatar, F. (2012). "Daily variability of river concentrations and fluxes: indicators based on the segmentation of the rating curve." *Hydrological Processes*, 26, 1188-1207.

Mills, D. K., Greenwood, N., Kroger, S., Devlin, M., Sivyer, D. B., Pearce, D., Cutchey, S., and Malcolm, S. J. (2005). "New approaches to improve the detection of eutrophication in UK coastal waters." *Environmental research, engineering and management*, 2(32), 36-42.

Monteagudo, L., Moreno, J. L., and Picazo, F. (2012). "River eutrophication: Irrigated vs. non-irrigated agriculture through different spatial scales." *Water Research*, 46, 2759-2771.

Mullin, M. M., Stewart, E. F., and Fuglister, F. J. (1975). "Ingestion by the planktonic grazers as a function of concentration of food." *Limnology and Oceanography*, 20, 259-262.

Pennak, R. W. (1989). *Fresh-water invertebrates of the United States: Protozoa to Mollusca*, John Wiley and Sons, Inc.

Peters, R. H. e. a. (1984). "Impirical analysis of zooplankton filtering and feeding rates." *Limnology and Oceanography*, 29, 763-784.

Pontin, R. M. (1978). "A key to the freshwater planktonic and semiplanktonic rotifera of the British Isles." *Freshwater Biological Association*, 38.

Poulin, F. J., and Franks, P. J. S. (2010). "Size-structured planktonic ecosystems: constraints, controls and assembly instructions." *Journal of Plankton Research*, 32(8), 1121-1130.

Riemann, B. (1985). "Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria." *Applied and Environmental Microbiology*, 50(2), 187-193.

Rolland S. Fulton, I. (1988). "Grazing on filamentous algae by herbivorous zooplankton." *Freshwater Biology*, 20, 263-271.

Roman, M. R., Ashton, K. A., and Gauzens, A. L. (1988). "Day/night differences in the grazing impact of marine copepods." *Hydrobiologia*, 167, 21-30.

Sanderson, B. G., Redden, A. M., and Evans, K. (2012). "Grazing constants are not constant: Microzooplankton grazing is a function of phytoplankton production in an Australian lagoon." *Estuaries and Coasts*, 35(5), 1270-1284.

Scheffer, M., Hosper, S. H., Meijer, M.-L., Moss, B., and Jeppesen, E. (1993). "Alternative equilibria in shallow lakes." *Trends in Ecology and Evolution*, 8(8), 275-279.

Sherr, B. F., Sherr, E. B., and Fallon, R. D. (1987). "Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory." *Applied and Environmental Microbiology*, 53(5), 958-965.

Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., and Malcolm, S. (2007). "Defining and detecting undesirable disturbance in the context of marine eutrophication." *Marine Pollution Bulletin*, 55, 282-297.

Tijdens, M., Waal, D. B. V. D., Slovackova, H., Hoogveld, H. L., and Gons, H. J. (2008). "Estimates of bacterial and phytoplankton mortality caused by viral lysis and microzooplankton grazing in a shallow eutrophic lake." *Freshwater Biology*, 53, 1126-1141.

Wallace, R. L., and Snell, T. W. (1991). "Ecology and classification of North American freshwater invertebrates." *Ecology and Classification of North American Freshwater Invertebrates*, J. H. Thorp, and A. P. Covich, eds., Academic Press Inc., 187-248.

White, J. R., and Roman, M. R. (1991). "Measurement of zooplankton grazing using particles labelled in light and dark with [methyl-^{3}H] methylamine hydrochloride." *Marine Ecology Progress Series*, 71, 45-52.

Widdicombe, C. E., Eloire, D., Harbour, D., Harris, R. P., and Somerfield, P. J. (2010). "Long-term phytoplankton community dynamics in the Western English Channel." *Journal of Plankton Research*, 32(5), 643-655.

Yu, Z. S., Northup, R. R., and Dahlgren, R. A. (1994). "Deterimination of dissolved organic nitrogen using persulfate oxidation and conductimetric quantification of nitrate-nitrogen." *Communications in Soil Science and Plant Analysis*, 25, 3161-3169.

APPENDIX A. CHLOROPHYLL ANALYSIS

Table 7. Chlorophyll analysis for the June 29, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
06/29/12	0.2um, 20% RW, 24hr inc	1.7	29.9	1.5
06/29/12	0.2um, 60% RW, 24hr inc	1.6	62.8	9.9
06/29/12	0.2um, 60% RW, 24hr inc	1.6	77.5	12.1
06/29/12	100%RW, 24hr inc	1.7	130.0	6.0
06/29/12	100%RW, 24hr inc	1.8	111.3	-9.7
06/29/12	100%RW, 24hr dark inc	1.6	99.4	14.7
06/29/12	0.2um, 60% RW, 24hr inc	1.6	66.3	11.0
06/29/12	30kDa, 60%RW, 24 inc	1.6	68.5	17.2
06/29/12	0.2um, 20% RW, 24hr inc	1.7	26.2	-0.3
06/29/12	0.2um, 40% RW, 24hr inc	1.7	45.8	3.7
06/29/12	30kDa, 40%RW, 24hr dark inc	1.7	39.7	1.9
06/29/12	0.2um, 40% RW, 24hr inc	1.7	51.9	0.9
06/29/12	0.2um, 20% RW, 24hr inc	1.7	30.9	0.2
06/29/12	100%RW, no inc	1.5	92.0	27.6
06/29/12	100%RW, 24hr inc	1.7	83.8	-0.7
06/29/12	100%RW, 24hr inc	1.7	132.0	5.8
06/29/12	30kDa, 40%RW, 24hr inc	1.7	43.1	2.7
06/29/12	30kDa, 20%RW, 24hr inc	1.7	32.0	0.7
06/29/12	100%RW, no inc	1.6	101.6	16.2
06/29/12	30kDa, 40%RW, 24hr inc	1.6	52.0	4.0
06/29/12	30kDa, 60%RW, 24 inc	1.6	65.3	14.4
06/29/12	100%RW, 24hr inc	1.7	118.8	2.8
06/29/12	30kDa, 20%RW, 24hr inc	1.7	25.5	0.2
06/29/12	30kDa, 20%RW, 24hr dark inc	1.7	20.5	-1.1

Notes: "0.2um" signifies samples filtered through a 0.2-µm filter
"30kDa" signifies samples filtered through a 30-kDa filter
"% RW" signifies the percentage of unfiltered river water within the sample
"inc" signifies incubation
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 8. Chlorophyll analysis for the July 17, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
07/17/12	0.2-um 20%	1.8	19.7	-1.8
07/17/12	0.2-um 20%	1.8	18.8	-1.9
07/17/12	0.2-um 20%	1.8	18.9	-1.6
07/17/12	30-kDa 20%	1.8	17.2	-2.3
07/17/12	30-kDa 20%	1.6	75.3	7.3
07/17/12	30-kDa 20%	1.8	15.4	-1.8
07/17/12	0.2-um 40%	1.7	31.6	-1.0
07/17/12	0.2-um 40%	1.7	32.1	-1.2
07/17/12	0.2-um 40%	1.7	29.9	-0.8
07/17/12	30-kDa 40%	1.7	29.9	-1.1
07/17/12	30-kDa 40%	1.7	29.4	-1.0
07/17/12	30-kDa 40%	1.7	29.9	-0.7
07/17/12	0.2-um 60%	1.7	45.0	-0.5
07/17/12	0.2-um 60%	1.7	47.6	-0.1
07/17/12	0.2-um 60%	1.7	46.2	-0.2
07/17/12	30-kDa 60%	1.7	45.0	-0.2
07/17/12	30-kDa 60%	1.7	44.7	1.1
07/17/12	30-kDa 60%	1.5	30.7	16.1
07/17/12	100% Initial	1.6	68.9	5.2
07/17/12	100% Initial	1.6	68.9	8.7
07/17/12	100% After	1.7	104.6	-5.5
07/17/12	100% After	1.7	87.2	-5.4
07/17/12	100% After	1.7	85.7	-5.3

Notes: "0.2-um" signifies samples filtered through a 0.2-µm filter
"30-kDa" signifies samples filtered through a 30-kDa filter
"%" signifies the percentage of unfiltered river water in a sample
"After" signifies post-incubation samples
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 9. Chlorophyll analysis for the July 31, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
07/31/12	WGNS T0	1.5	31.5	13.1
07/31/12	WGNS T0	1.5	33.6	12.2
07/31/12	WOG T0	1.5	30.7	12.9
07/31/12	WOG T0	1.5	31.1	11.9
07/31/12	WGS T0	1.5	34.9	11.9
07/31/12	WGS T0	1.5	34.7	13.0
07/31/12	CGNS T0	1.6	62.5	13.7
07/31/12	CGNS T0	1.6	69.5	13.6
07/31/12	WGNS T3	1.5	31.5	12.2
07/31/12	WGNS T3	1.5	33.4	11.5
07/31/12	WOG T3	1.5	30.1	10.3
07/31/12	WOG T3	1.5	30.4	12.3
07/31/12	WGS T3	1.5	35.6	12.8
07/31/12	WGS T3	1.5	39.1	10.9
07/31/12	WGNS T6	1.5	33.3	13.4
07/31/12	WGNS T6	1.5	31.5	11.1
07/31/12	WOG T6	1.5	31.0	12.1
07/31/12	WOG T6	1.5	27.7	12.4
07/31/12	WGS T6	1.5	33.1	12.1
07/31/12	WGS T6	1.5	32.5	12.1
07/31/12	WGNS T24	1.4	22.6	15.3
07/31/12	WGNS T24	1.4	21.6	16.0
07/31/12	WOG T24	1.4	19.7	14.6
07/31/12	WOG T24	1.4	21.4	15.3
07/31/12	WGS T24	1.4	21.3	13.4
07/31/12	WGS T24	1.5	27.9	14.3
07/31/12	CGNS T24	1.5	54.2	15.7
07/31/12	CGNS T24	1.5	41.9	13.2
07/31/12	WGNS T30	1.4	21.9	15.6
07/31/12	WGNS T30	1.4	21.5	16.6
07/31/12	WOG T30	1.4	16.5	15.7
07/31/12	WOG T30	1.3	16.1	16.6
07/31/12	WGS T30	1.4	19.7	14.7
07/31/12	WGS T30	1.4	18.5	16.8
07/31/12	CGNS T30	1.5	41.3	18.8
07/31/12	CGNS T30	1.5	40.0	18.1
07/31/12	WGNS T54	1.3	12.6	20.2
07/31/12	WGNS T54	1.3	12.6	22.2
07/31/12	WOG T54	1.3	11.7	19.4
07/31/12	WOG T54	1.3	11.8	16.2
07/31/12	WGS T54	1.2	10.4	19.6
07/31/12	WGS T54	1.3	13.0	23.3
07/31/12	0.2-um 40%	1.6	11.7	2.6
07/31/12	0.2-um 40%	1.5	10.3	2.8
07/31/12	0.2-um 40%	1.5	11.1	3.8
07/31/12	30-kDa 40%	1.6	14.0	3.5
07/31/12	30-kDa 40%	1.6	11.9	2.7
07/31/12	30-kDa 40%	1.6	11.0	2.6

Notes: "WGNS" signifies samples with grazers where mixing occurred
"WOG" signifies samples where grazers were removed
"WGS" signifies samples with grazers where settling was allowed
"CGNS" signifies samples where grazers were concentrated and mixing occurred

"T" signifies a time in hours
"0.2-um" signifies samples filtered through a 0.2-µm filter
"30-kDa" signifies samples filtered through a 30-kDa filter
"%" signifies the percentage of unfiltered river water in a sample
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 10. Chlorophyll analysis for the August 13, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
08/13/12	WGNS T0	1.6	36.7	3.9
08/13/12	WGNS T0	1.6	36.9	3.3
08/13/12	WOG T0	1.6	36.7	3.6
08/13/12	WOG T0	1.6	36.2	3.9
08/13/12	WGS T0	1.6	36.8	4.0
08/13/12	CGNS T0	1.6	40.1	3.3
08/13/12	CGNS T0	1.6	39.9	4.4
08/13/12	WGNS T3	1.6	35.6	4.6
08/13/12	WGNS T3	1.6	36.4	4.2
08/13/12	WOG T3	1.6	35.7	3.3
08/13/12	WOG T3	1.6	36.7	3.2
08/13/12	WGS T3	1.6	37.8	4.1
08/13/12	WGS T3	1.6	34.6	3.5
08/13/12	WGNS T6	1.6	34.2	4.8
08/13/12	WGNS T6	1.6	34.3	4.9
08/13/12	WOG T6	1.6	34.5	4.2
08/13/12	WOG T6	1.6	34.4	5.2
08/13/12	WGS T6	1.6	34.8	5.6
08/13/12	WGS T6	1.6	32.6	5.4
08/13/12	WGNS T24	1.5	24.5	8.6
08/13/12	WGNS T24	1.5	23.9	8.6
08/13/12	WOG T24	1.5	24.2	8.3
08/13/12	WOG T24	1.5	24.1	7.4
08/13/12	WGS T24	1.5	21.9	8.7
08/13/12	WGS T24	1.5	22.9	8.3
08/13/12	CGNS T24	1.5	25.8	10.6
08/13/12	CGNS T24	1.5	26.0	9.2
08/13/12	WGNS T30	1.5	19.8	10.1
08/13/12	WGNS T30	1.5	18.8	9.6
08/13/12	WOG T30	1.5	19.4	7.7
08/13/12	WOG T30	1.5	18.3	7.2
08/13/12	WGS T30	1.5	16.7	9.5
08/13/12	WGS T30	1.5	16.6	8.8
08/13/12	CGNS T30	1.5	21.3	10.0
08/13/12	CGNS T30	1.5	21.2	10.1
08/13/12	0.2um 40%	1.7	13.6	0.8
08/13/12	0.2um 40%	1.6	13.2	1.1
08/13/12	0.2um 40%	1.7	13.3	0.2
08/13/12	30kDa 40%	1.7	13.1	0.0
08/13/12	30kDa 40%	1.7	12.3	0.5

Notes: "WGNS" signifies samples with grazers where mixing occurred
"WOG" signifies samples where grazers were removed

"WGS" signifies samples with grazers where settling was allowed
"CGNS" signifies samples where grazers were concentrated and mixing occurred
"T" signifies a time in hours
"0.2-um" signifies samples filtered through a 0.2-µm filter
"30-kDa" signifies samples filtered through a 30-kDa filter
"%" signifies the percentage of unfiltered river water in a sample
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 11. Chlorophyll analysis for the August 20, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
08/20/12	No Zoo T0	1.6	4.7	1.0
08/20/12	No Zoo T0	1.6	4.2	1.3
08/20/12	1X T0	1.6	5.7	1.2
08/20/12	1X T0	1.6	5.2	0.8
08/20/12	7.5X T0	1.6	7.5	0.9
08/20/12	7.5X T0	1.7	9.9	1.0
08/20/12	15X T0	1.6	10.5	1.3
08/20/12	15X T0	1.7	13.3	0.8
08/20/12	30X T0	1.6	11.4	1.3
08/20/12	30X T0	1.6	12.6	1.3
08/20/12	No Zoo T6 light	1.7	7.6	-0.2
08/20/12	No Zoo T6 light	1.8	7.2	-1.1
08/20/12	1X T6 light	1.8	8.0	-0.8
08/20/12	1X T6 light	1.7	8.6	-0.4
08/20/12	7.5X T6 light	1.8	8.8	-0.5
08/20/12	7.5X T6 light	1.7	8.9	-0.4
08/20/12	15X T6 light	1.7	11.5	-0.1
08/20/12	15X T6 light	1.7	11.9	-0.4
08/20/12	30X T6 light	1.7	16.3	-0.6
08/20/12	30X T6 light	1.7	16.0	0.2
08/20/12	No Zoo T6 dark	1.5	5.2	1.5
08/20/12	1X T6 dark	1.5	5.4	1.8
08/20/12	7.5X T6 dark	1.6	11.3	1.2
08/20/12	15X T6 dark	1.6	15.0	1.4
08/20/12	30X T6 dark	1.6	11.2	1.5

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
"T0" signifies initial samples
"T6" signifies post-incubation (final) samples
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 12. Chlorophyll analysis for the September 21, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
09/21/12	No Zoo Initial	1.6	8.3	1.7
09/21/12	1X Initial	1.6	9.0	1.9
09/21/12	7.5X Initial	1.6	9.5	2.0
09/21/12	15X Initial	1.6	11.4	2.1
09/21/12	22.5X Initial	1.6	12.9	2.5
09/21/12	30X Initial	1.6	14.2	2.9
09/21/12	No Zoo Light	1.7	12.5	0.7
09/21/12	No Zoo Light	1.7	12.4	0.1
09/21/12	1X Light	1.7	12.3	0.7
09/21/12	1X Light Shaded	1.8	13.1	-0.9
09/21/12	1X Light Shaded	1.8	13.2	-1.1
09/21/12	7.5X Light	1.7	13.4	0.9
09/21/12	7.5X Light	1.7	14.0	0.8
09/21/12	15X Light	1.7	12.2	0.4
09/21/12	15X Light	1.7	12.3	0.3
09/21/12	22.5X Light	1.6	12.5	1.5
09/21/12	22.5X Light Shaded	1.6	13.1	1.5
09/21/12	30X Light	1.6	9.5	2.2
09/21/12	30X Light	1.6	10.3	1.7
09/21/12	No Zoo Dark	1.6	7.5	1.6
09/21/12	1X Dark	1.6	7.4	2.1
09/21/12	7.5X Dark	1.5	8.5	2.9
09/21/12	15X Dark	1.5	8.6	3.1
09/21/12	22.5X Dark	1.5	9.8	3.2
09/21/12	30X Dark	1.5	10.3	3.8

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
Samples without the "initial" label are post-incubation samples
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 13. Chlorophyll analysis for the October 22, 2012 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
10/22/12	No Zoo Initial	1.7	4.9	0.1
10/22/12	No Zoo Initial	1.6	5.2	0.9
10/22/12	1X Initial	1.7	5.1	0.0
10/22/12	1X Initial	1.7	5.3	0.0
10/22/12	7.5X Initial	1.7	5.7	0.1
10/22/12	7.5X Initial	1.7	5.8	0.4
10/22/12	15X Initial	1.7	7.6	0.4
10/22/12	15X Initial	1.7	8.5	0.2
10/22/12	22.5X Initial	1.6	8.1	0.6
10/22/12	30X Initial	1.6	9.5	0.8
10/22/12	30X Initial	1.6	10.2	1.6
10/22/12	No Zoo	1.6	7.2	1.1
10/22/12	No Zoo	1.7	6.3	0.5
10/22/12	1X	1.6	6.3	0.6
10/22/12	1X	1.7	6.3	0.1
10/22/12	7.5X	1.6	5.9	0.6
10/22/12	7.5X	1.6	6.2	0.8
10/22/12	15X	1.6	6.7	0.9
10/22/12	15X	1.6	7.1	1.6
10/22/12	22.5X	1.6	7.5	1.7
10/22/12	22.5X	1.6	7.1	1.6
10/22/12	30X	1.6	8.4	1.7
10/22/12	30X	1.6	9.0	2.1
10/22/12	No Zoo Dark	1.5	4.4	2.0
10/22/12	1X Dark	1.4	3.6	2.5
10/22/12	7.5X Dark	1.4	4.4	3.6
10/22/12	15X Dark	1.4	4.7	3.6
10/22/12	22.5X Dark	1.4	5.2	3.3
10/22/12	30X Dark	1.4	6.3	4.1

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
Samples without the "initial" label are post-incubation samples
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 14. Chlorophyll analysis for the April 18, 2013 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
04/18/13	No Zoo Initial	1.5	4.7	1.5
04/18/13	1X Initial	1.7	6.5	-0.1
04/18/13	7.5X Initial	1.7	8.7	0.4
04/18/13	15X Initial	1.7	12.9	0.7
04/18/13	22.5X Initial	1.7	17.1	0.3
04/18/13	30X Initial	1.7	21.1	1.5
04/18/13	No Zoo Light	1.7	8.2	0.2
04/18/13	No Zoo Light	1.6	8.1	0.8
04/18/13	No Zoo Dark	1.6	2.3	0.3
04/18/13	No Zoo Dark	1.5	4.2	1.7
04/18/13	1X Light	1.7	9.1	-0.4
04/18/13	1X Light	1.6	8.6	1.0
04/18/13	1X Dark	1.5	5.3	2.2
04/18/13	1X Dark	1.5	4.9	1.8
04/18/13	7.5X Light	1.6	11.5	1.2
04/18/13	7.5X Light	1.7	12.0	0.5
04/18/13	7.5X Dark	1.5	7.8	2.5
04/18/13	7.5X Dark	1.5	6.7	3.1
04/18/13	15X Light	1.7	16.4	0.0
04/18/13	15X Light	1.6	16.0	1.6
04/18/13	15X Dark	1.5	9.9	4.1
04/18/13	15X Dark	1.5	10.0	3.2
04/18/13	22.5X Light	1.7	17.4	1.0
04/18/13	22.5X Light	1.7	20.5	0.9
04/18/13	22.5X Dark	1.5	12.6	4.8
04/18/13	22.5X Dark	1.5	14.2	4.4
04/18/13	30X Light	1.7	24.3	1.7
04/18/13	30X Light	1.7	23.6	1.3
04/18/13	30X Dark	1.5	16.1	5.5
04/18/13	30X Dark	1.5	14.0	6.1

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
Samples without the "initial" label are post-incubation samples
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 15. Chlorophyll analysis for the June 7, 2013 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
06/07/13	No Zoo Initial	1.5	10.6	3.1
06/07/13	1X Initial	1.6	14.4	1.9
06/07/13	7.5X Initial	1.6	19.9	2.3
06/07/13	15X Initial	1.6	26.2	3.0
06/07/13	22.5X Initial	1.6	31.7	3.4
06/07/13	30X Initial	1.6	38.5	4.2
06/07/13	No Zoo Light	1.7	37.8	1.0
06/07/13	No Zoo Light	1.7	41.7	0.0
06/07/13	No Zoo Dark	1.6	9.9	2.1
06/07/13	No Zoo Dark	1.5	9.9	3.1
06/07/13	1X Light	1.7	43.8	1.4
06/07/13	1X Light	1.7	43.6	0.8
06/07/13	1X Dark	1.6	13.2	3.3
06/07/13	1X Dark	1.5	10.1	2.7
06/07/13	7.5X Light	1.7	25.6	0.5
06/07/13	7.5X Light	1.7	20.5	1.2
06/07/13	7.5X Dark	1.6	14.6	3.2
06/07/13	7.5X Dark	1.6	14.1	1.7
06/07/13	15X Light	1.7	28.9	-0.5
06/07/13	15X Light	1.8	25.2	-1.6
06/07/13	15X Dark	1.5	15.1	8.3
06/07/13	15X Dark	1.7	18.8	0.6
06/07/13	22.5X Light	1.7	39.2	0.1
06/07/13	22.5X Light	1.7	34.6	0.3
06/07/13	22.5X Dark	1.7	28.9	0.0
06/07/13	22.5X Dark	1.7	26.8	0.1
06/07/13	30X Light	1.7	44.4	-1.0
06/07/13	30X Light	1.7	44.9	-1.0
06/07/13	30X Dark	1.7	33.7	-0.3
06/07/13	30X Dark	1.7	33.3	0.0

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
Samples without the "initial" label are post-incubation samples
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 16. Chlorophyll analysis for the June 18, 2013 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
06/18/13	No Zoo UF INITIAL	2.0	6.6	-2.0
06/18/13	No Zoo F INITIAL	2.2	6.7	-2.8
06/18/13	1X UF INITIAL	2.6	10.0	-5.5
06/18/13	1X F INITIAL	3.1	7.2	-4.8
06/18/13	7.5X UF INITIAL	1.8	23.4	-4.1
06/18/13	7.5X F INITIAL	1.7	13.8	0.3
06/18/13	15X UF INITIAL	1.8	47.2	-4.9
06/18/13	15X F INITIAL	1.9	20.0	-4.2
06/18/13	22.5X UF INITIAL	1.8	58.8	-4.0
06/18/13	22.5X F INITIAL	1.8	29.5	-2.8
06/18/13	30X UF INITIAL	1.7	78.8	-2.0
06/18/13	30X F INITIAL	1.8	36.6	-3.3
06/18/13	No Zoo Final Dark	1.8	5.8	-0.8
06/18/13	No Zoo Final Dark	1.7	6.5	0.1
06/18/13	No Zoo Final Light	1.7	15.8	-1.0
06/18/13	No Zoo Final Light	1.7	19.4	-1.0
06/18/13	1X Final Dark UF 1	1.8	9.8	-1.0
06/18/13	1X Final Dark UF 2	1.9	9.5	-1.8
06/18/13	1X Final Dark F 1	1.7	8.1	-0.3
06/18/13	1X Final Dark F 2	1.9	6.3	-1.5
06/18/13	1X Final Light UF 1	1.9	25.5	-5.0
06/18/13	1X Final Light UF 2	1.8	21.2	-3.1
06/18/13	1X Final Light F 1	1.9	17.3	-3.5
06/18/13	1X Final Light F 2	1.8	16.1	-2.4
06/18/13	7.5X Final Dark UF 1	1.8	16.7	-1.7
06/18/13	7.5X Final Dark UF 2	1.7	23.4	-1.2
06/18/13	7.5X Final Dark F 1	1.7	12.4	-0.8
06/18/13	7.5X Final Dark F 2	1.8	11.5	-1.4
06/18/13	7.5X Final Light UF 1	1.7	36.3	-1.6
06/18/13	7.5X Final Light UF 2	1.8	34.4	-2.8
06/18/13	7.5X Final Light F 1	1.8	22.2	-1.6
06/18/13	7.5X Final Light F 2	1.8	19.9	-3.3
06/18/13	15X Final Dark UF 1	1.7	42.1	-0.9
06/18/13	15X Final Dark UF 2	1.7	30.8	-0.1
06/18/13	15X Final Dark F 1	1.8	18.9	-1.7
06/18/13	15X Final Dark F 2	1.7	18.1	0.1
06/18/13	15X Final Light UF 1	1.7	62.3	-1.0
06/18/13	15X Final Light UF 2	1.7	55.4	2.0
06/18/13	15X Final Light F 1	1.7	31.0	-1.3
06/18/13	15X Final Light F 2	1.7	29.9	0.2
06/18/13	22.5X Final Dark UF	1.7	46.2	2.4
06/18/13	22.5X Final Dark F	1.7	25.9	0.7
06/18/13	22.5X Final Light UF	1.7	72.5	4.3
06/18/13	22.5X Final Light F	1.7	42.6	0.8
06/18/13	30X Final Dark UF 1	1.7	57.3	0.5
06/18/13	30X Final Dark UF 2	1.7	78.8	5.2
06/18/13	30X Final Dark F 1	1.7	32.5	0.3
06/18/13	30X Final Dark F 2	1.7	33.7	0.3
06/18/13	30X Final Light UF 1	1.7	86.5	3.6
06/18/13	30X Final Light UF 2	1.7	90.3	2.1
06/18/13	30X Final Light F 1	1.7	43.2	-0.2
06/18/13	30X Final Light F 2	1.7	42.8	-1.5

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"UF" signifies samples where pre-screening was not performed
"F" signifies samples where pre-screening was performed
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

Table 17. Chlorophyll analysis for the July 25, 2013 grazing study.

Study Date	Sample ID	$664_b/665_a$	Chla (ug/L)	Pha (ug/L)
07/25/13	No Zoo INITIAL	1.4	5.9	3.4
07/25/13	No Zoo INITIAL	1.3	5.1	5.8
07/25/13	1X UF INITIAL	1.8	13.0	-0.8
07/25/13	1X F INITIAL	1.8	12.1	-0.8
07/25/13	7.5X UF INITIAL	1.7	22.5	-0.3
07/25/13	7.5X F INITIAL	1.6	15.5	2.4
07/25/13	15X UF INITIAL	1.7	38.1	0.8
07/25/13	15X F INITIAL	1.7	26.3	0.2
07/25/13	22.5X UF INITIAL	1.7	46.1	-1.4
07/25/13	22.5X F INITIAL	1.7	31.0	0.9
07/25/13	30X UF INITIAL	1.7	60.3	-1.2
07/25/13	30X F INITIAL	1.7	36.8	0.0
07/25/13	No Zoo Final Dark	1.5	5.0	1.9
07/25/13	No Zoo Final Dark	1.6	4.9	0.9
07/25/13	No Zoo Final Dark	1.5	5.5	2.3
07/25/13	No Zoo Final Dark	1.5	4.4	1.4
07/25/13	No Zoo Final Light	1.8	14.3	-1.4
07/25/13	No Zoo Final Light	1.7	16.6	-0.7
07/25/13	No Zoo Final Light	1.7	15.6	-0.3
07/25/13	No Zoo Final Light	1.8	12.2	-1.1
07/25/13	1X Final Light UF 1	1.7	26.9	-0.2
07/25/13	1X Final Light UF 2	1.7	24.1	-1.2
07/25/13	1X Final Dark UF 1	1.6	12.4	1.5
07/25/13	1X Final Dark UF 2	1.7	10.0	0.1
07/25/13	1X Final Light F 1	1.8	22.5	-2.3
07/25/13	1X Final Light F 2	1.8	21.7	-2.4
07/25/13	1X Final Dark F 1	1.8	9.4	-0.9
07/25/13	1X Final Dark F 2	1.7	8.6	-0.1
07/25/13	7.5X Final Light UF 1	1.9	23.1	-4.9
07/25/13	7.5X Final Light UF 2	2.0	25.0	-6.4
07/25/13	7.5X Final Dark UF 1	1.8	18.2	-2.1
07/25/13	7.5X Final Dark UF 2	1.8	17.7	-1.2
07/25/13	7.5X Final Light F 1	2.0	14.8	-4.4
07/25/13	7.5X Final Light F 2	2.1	12.9	-4.8
07/25/13	7.5X Final Dark F 1	1.8	13.0	-1.5
07/25/13	15X Final Light UF 1	1.8	35.1	-4.7
07/25/13	15X Final Light UF 2	1.8	39.2	-4.0
07/25/13	15X Final Dark UF 1	1.8	27.6	-2.3
07/25/13	15X Final Dark UF 2	1.7	25.7	1.4
07/25/13	15X Final Light F 1	1.8	22.2	-3.6

07/25/13	15X Final Light F 2	1.8	20.6	-3.3
07/25/13	15X Final Dark F 1	1.8	17.8	-2.6
07/25/13	15X Final Dark F 2	1.8	15.5	-1.6
07/25/13	22.5X Final Light UF 1	1.8	50.0	-6.0
07/25/13	22.5X Final Light UF 2	1.8	50.9	-4.4
07/25/13	22.5X Final Dark UF 1	1.7	33.9	-1.1
07/25/13	22.5X Final Dark UF 2	1.7	30.3	1.2
07/25/13	22.5X Final Light F 1	1.8	26.0	-3.4
07/25/13	22.5X Final Light F 2	1.8	26.0	-4.7
07/25/13	22.5X Final Dark F 1	1.7	18.3	-1.2
07/25/13	22.5X Final Dark F 2	1.8	21.3	-1.6
07/25/13	30X Final Light UF 1	1.8	50.8	-4.1
07/25/13	30X Final Light UF 2	1.7	59.1	-3.6
07/25/13	30X Final Dark UF 1	1.7	38.3	-2.2
07/25/13	30X Final Dark UF 2	1.7	39.2	3.1
07/25/13	30X Final Light F 1	1.8	29.0	-4.7
07/25/13	30X Final Light F 2	1.8	27.9	-4.9
07/25/13	30X Final Dark F 1	1.8	26.4	-2.7
07/25/13	30X Final Dark F 2	1.8	22.7	-2.6

Notes: "No Zoo" signifies samples where zooplankton was removed
"—X" signifies the concentration factor for a sample
"light" signifies clear sample bottled microcosms
"dark" signifies samples covered to block sunlight from penetrating the microcosm
"UF" signifies samples where pre-screening was not performed
"F" signifies samples where pre-screening was performed
"664_b" signifies wavelength 664-nm before acidification
"665_a" signifies wavelength 665-nm after acidification

APPENDIX B. ZOOPLANKTON ANALYSIS

Table 18. Zooplankton analysis for the April 18, 2013 grazing study.

Study Date	Sample ID	Total zoo (ug/L)	Rotifer (ug/L)	Copepod (ug/L)	Cladoceran (ug/L)
04/18/13	1X Initial	87.3	7.3	80.0	0.0
04/18/13	7.5X Initial	523.7	205.6	318.0	0.0
04/18/13	15X Initial	706.0	78.2	430.4	197.4
04/18/13	22.5X Initial	1049.6	84.9	565.6	399.1
04/18/13	30X Initial	1595.7	63.1	1472.5	60.2
04/18/13	60X Initial	2413.2	114.8	1787.6	510.8
04/18/13	60X Initial	3353.2	161.1	2857.5	334.6
04/18/13	1X Final	0.0	0.0	0.0	0.0
04/18/13	7.5X Final	772.6	27.0	745.6	0.0
04/18/13	15X Final	1082.1	201.5	724.4	156.2
04/18/13	22.5X Final	792.2	15.2	628.1	148.9
04/18/13	30X Final	1164.8	55.4	15.2	199.6

Table 19. Zooplankton analysis for the June 7, 2013 grazing study.

Study Date	Sample ID	Total zoo (ug/L)	Rotifer (ug/L)	Copepod (ug/L)	Cladoceran (ug/L)
06/07/13	1X Initial	0.6	0.6	0.0	0.0
06/07/13	7.5X Initial	1072.4	45.8	482.0	544.6
06/07/13	15X Initial	1709.9	61.4	1412.7	235.8
06/07/13	22.5X Initial	2701.4	17.4	1957.0	727.0
06/07/13	30X Initial	4969.5	74.6	2872.1	2022.8
06/07/13	60X	11133.3	0.5	80.0	0.0
06/07/13	60X	11633.3	133.4	6294.6	4705.3
06/07/13	1X Final	80.5	167.5	7317.3	4148.5
06/07/13	7.5X Final	908.9	52.7	1246.3	1241.1
06/07/13	15X Final	1995.8	13.1	213.9	681.9
06/07/13	22.5X Final	2540.0	27.6	1049.9	918.3
06/07/13	30X Final	4707.6	3.2	2307.8	2396.6

Table 20. Zooplankton analysis for the June 18, 2013 grazing study.

Study Date	Sample ID	Total zoo (ug/L)	Rotifer (ug/L)	Copepod (ug/L)	Cladoceran (ug/L)
06/18/13	1X Initial	131.3	0.2	40.0	91.2
06/18/13	7.5X Initial	118.9	1.1	40.0	77.8
06/18/13	15X Initial	502.6	9.4	360.0	133.2
06/18/13	22.5X Initial	831.1	13.4	360.0	457.7
06/18/13	30X Initial	1399.0	16.0	954.5	428.6
06/18/13	60X Initial	3783.0	13.2	1379.6	2390.2
06/18/13	60X Initial	3704.4	13.6	2472.7	1218.1
06/18/13	1X Final	120.6	0.6	120.0	0.0
06/18/13	7.5X Final	400.9	0.8	240.0	160.1
06/18/13	15X Final	300.2	20.2	280.0	0.0
06/18/13	22.5X Final	260.4	20.4	240.0	0.0
06/18/13	30X Final	719.8	12.4	419.8	287.6

Table 21. Zooplankton analysis for the July 25, 2013 grazing study.

Study Date	Sample ID	Total zoo (ug/L)	Rotifer (ug/L)	Copepod (ug/L)	Cladoceran (ug/L)
07/25/13	1X Initial	139.0	4.0	40.0	94.9
07/25/13	7.5X Initial	1812.4	12.2	529.1	1271.2
07/25/13	15X Initial	3560.3	20.2	635.1	2905.0
07/25/13	22.5X Initial	6059.0	110.0	837.9	5111.0
07/25/13	30X Initial	8184.5	42.8	1670.4	6471.3
07/25/13	1X Final	518.4	2.5	220.6	295.3
07/25/13	7.5X Final	1983.2	10.0	174.8	1798.4
07/25/13	15X Final	3455.3	34.0	999.5	2421.7
07/25/13	22.5X Final	2878.2	54.3	508.1	2315.9
07/25/13	30X Final	6128.4	34.4	520.0	5573.9
07/25/13	60X Initial	15284.6	113.4	3856.4	11314.9

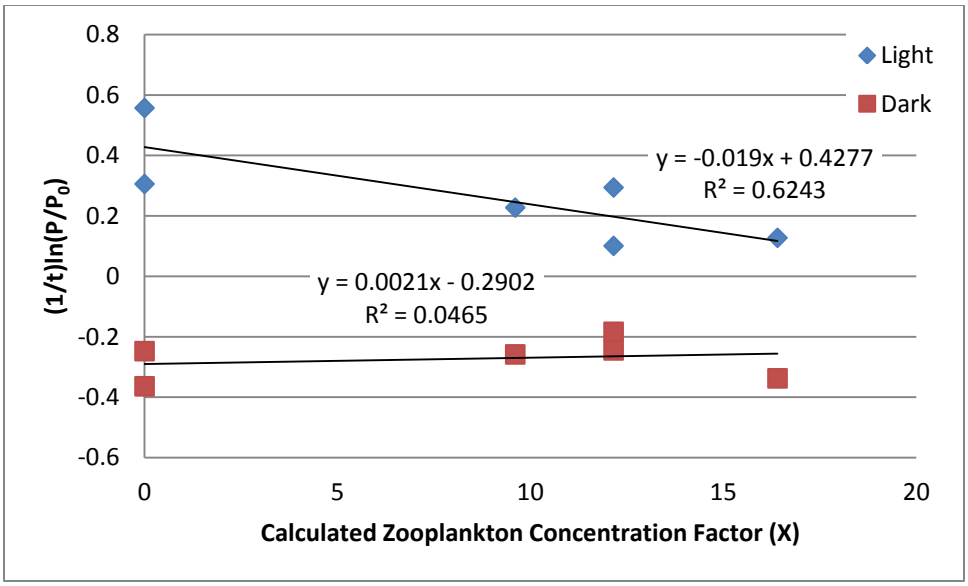

Figure 30. Fitted linear regression for the April 18, 2013 grazing study light and dark microcosms using calculated zooplankton concentration factors from final zooplankton densities.

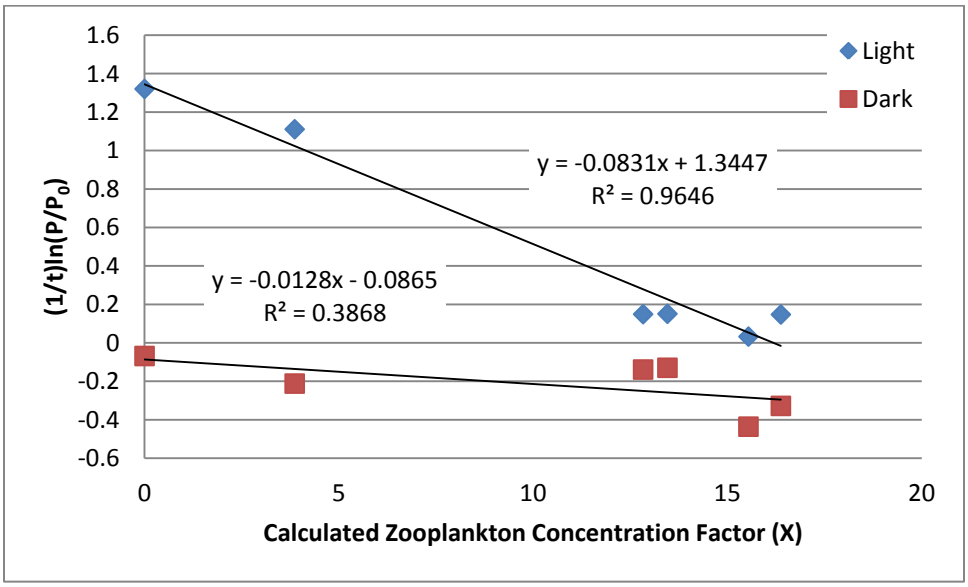

Figure 31. Fitted linear regression for the June 7, 2013 grazing study light and dark microcosms using calculated zooplankton concentration factors from final zooplankton densities.

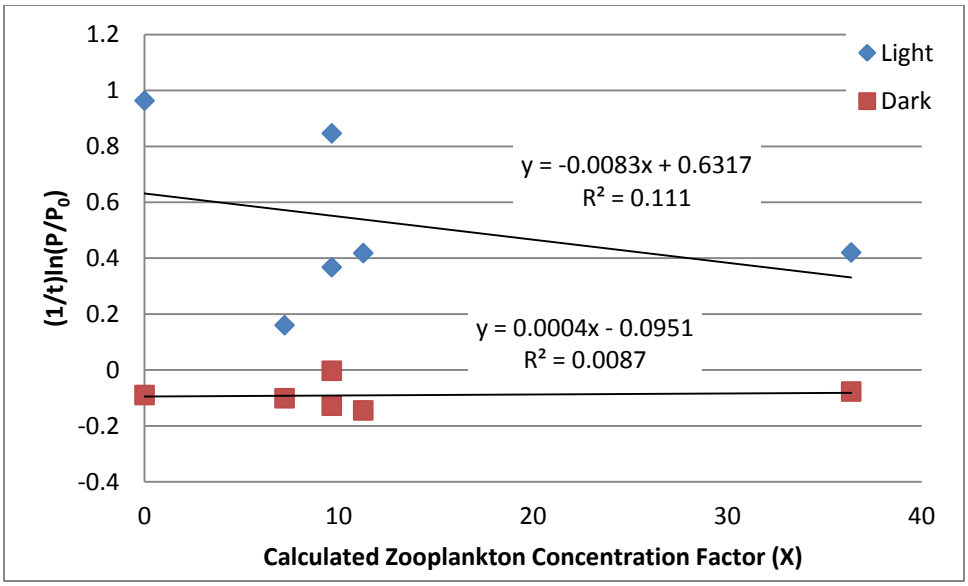

Figure 32. Fitted linear regression for the June 18, 2013 grazing study light and dark microcosms using calculated zooplankton concentration factors from final zooplankton densities.

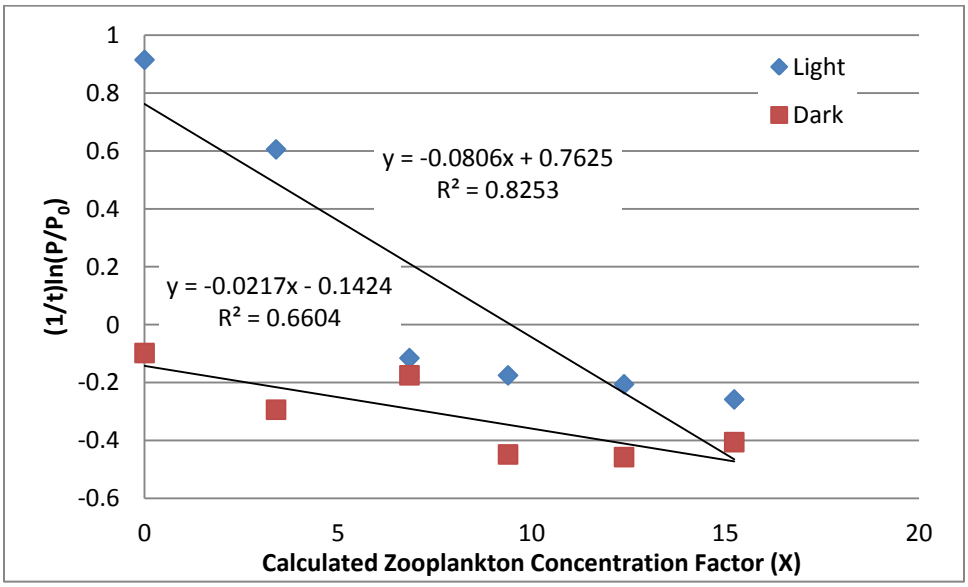

Figure 33. Fitted linear regression for the July 25, 2013 grazing study light and dark microcosms using calculated zooplankton concentration factors from final zooplankton densities.

APPENDIX C. NUTRIENT ANALYSIS

Table 22. Nutrient analysis for the July 17, 2012 grazing study.

Study Date	Sample ID	SiO2-Si (mg/L)	Total P (mg/L)	Dissolved PO4-P (mg/L)	Dissolved NO3-N (ug/L)	Total NH3/NH4-N (ug/L)	Dissolved NO2-N (ug/L)
07/17/12	TB Sample	1.2	0.3	0.2	821.0	11.0	31.0

Table 23. Nutrient analysis for the October 22, 2012 grazing study.

Study Date	Sample ID	Dissolved PO4-P (mg/L)	Total P (mg/L)	Dissolved inorganic N (ug/L)	Total NH3/NH4-N (ug/L)	Dissolved NO2-N (ug/L)	Dissolved NO3-N (ug/L)
10/22/12	1X Initial	0.4	0.3	2232.0	65.0	30.9	2136.1
10/22/12	1X Final	0.3	0.3	2286.2	49.3	30.9	2206.0
10/22/12	7.5X Final	0.3	0.4	2232.1	57.7	31.4	2143.0
10/22/12	15X Final	0.3	0.3	2665.6	73.5	32.0	2560.1
10/22/12	22.5X Final	0.3	0.3	2432.9	86.6	30.7	2315.6
10/22/12	30X Final	0.3	0.4	2349.2	2.8	31.0	2315.4
10/22/12	No Zoo Final	0.3	0.3	2617.6	49.4	32.1	2536.1

Table 24. Nutrient analysis for the April 18, 2013 grazing study.

Study Date	Sample ID	SiO2-Si (mg/L)	Dissolved PO4-P (mg/L)	Total P (mg/L)	Dissolved NO2-N (µg/L)	NH4-N (µg/L)	NO3-N + NO2-N (µg/L)	Total N (µg/L)
04/18/13	No Zoo Initial	6.2	0.3	0.4	29.2	74.7	2965.1	3340.3
04/18/13	1X Initial	6.3	0.3	0.4	30.0	70.6	2853.5	3655.2
04/18/13	7.5X Initial	6.2	0.4	0.4	29.3	79.2	2990.2	3581.8
04/18/13	15X Initial	6.2	0.3	0.4	30.1	78.7	3061.7	3617.2
04/18/13	22.5X Initial	6.3	0.3	0.4	30.4	82.9	3207.7	3985.1
04/18/13	30X Initial	6.2	0.3	0.4	31.2	76.7	2963.0	3456.9
04/18/13	No Zoo Final	6.2	0.3	0.4	29.3	50.2	3195.1	3543.4
04/18/13	1X Final	6.2	0.3	0.4	28.6	45.9	3055.4	3734.5
04/18/13	7.5X Final	6.1	0.3	0.4	29.8	48.7	3149.1	3605.8
04/18/13	15X Final	6.0	0.4	0.4	30.6	52.4	2814.0	3511.8
04/18/13	22.5X Final	5.7	0.3	0.4	28.1	49.1	3144.2	3873.0
04/18/13	30X Final	5.7	0.4	0.4	31.1	57.0	3021.9	3710.1

Table 25. Nutrient analysis for the June 7, 2013 grazing study.

Study Date	Sample ID	SiO2-Si (mg/L)	Dissolved PO4-P (mg/L)	Total P (mg/L)	Dissolved NO2-N (µg/L)	NH4-N (µg/L)	NO3-N + NO2-N (µg/L)	Total N (µg/L)
06/07/13	No Zoo Initial	4.7	0.3	0.3	24.7	26.1	1557.1	2242.4
06/07/13	1X Initial	4.7	0.3	0.3	24.8	23.8	1562.5	2404.5
06/07/13	7.5X Initial	4.8	0.3	0.3	23.6	36.9	1575.1	2728.4
06/07/13	15X Initial	4.7	0.3	0.4	24.8	44.2	1571.0	2913.8
06/07/13	22.5X Initial	4.8	0.3	0.4	25.8	41.3	1552.4	2317.8
06/07/13	30X Initial	4.7	0.3	0.4	25.4	46.2	1559.6	2978.8
06/07/13	No Zoo Final	4.4	0.2	0.3		9.5	1574.7	2195.4
06/07/13	1X Final	4.4	0.2	0.3		23.4	1400.1	2508.6
06/07/13	7.5X Final	4.7	0.3	0.4		43.9	1573.0	3219.9
06/07/13	15X Final	4.7	0.3	0.4		96.3	1787.1	3094.9
06/07/13	22.5X Final	4.7	0.4	0.5		91.5	1747.4	2404.8
06/07/13	30X Final	4.7	0.3	0.6		234.2	1458.7	3283.0

Table 26. Nutrient analysis for the June 18, 2013 grazing study.

Study Date	Sample ID	SiO2-Si (mg/L)	Dissolved PO4-P (mg/L)	Total P (mg/L)	Dissolved NO2-N (µg/L)	NH4-N (µg/L)	NO3-N + NO2-N (µg/L)	Total N (µg/L)
06/18/13	No Zoo Initial	5.8	0.4	0.5	22.3	16.0	2086.6	2629.7
06/18/13	1X Initial	5.7	0.4	0.5	23.7	7.7	2122.7	2460.3
06/18/13	7.5X Initial	5.6	0.5	0.5	22.2	7.1	2004.7	2742.8
06/18/13	15X Initial	5.8	0.5	0.5	22.3	7.1	2067.3	3013.1
06/18/13	22.5X Initial	5.8	0.5	0.5	22.6	5.7	1975.4	2719.1
06/18/13	30X Initial	5.8	0.5	0.6	21.7	4.1	1986.3	2719.2
06/18/13	No Zoo Final	5.7	0.4	0.5		59.6	1848.5	2891.9
06/18/13	1X Final	5.7	0.4	0.5		40.1	1986.4	2894.5
06/18/13	7.5X Final	5.8	0.4	0.5		57.3	1954.5	2932.5
06/18/13	15X Final	5.7	0.4	0.5		44.4	1882.7	3428.7
06/18/13	22.5X Final	5.8	0.4	0.5		51.1	1965.9	3521.9
06/18/13	30X Final	5.7	0.4	0.6		52.2	1947.4	3457.8

Table 27. Nutrient analysis for the July 25, 2013 grazing study.

Study Date	Sample ID	SiO2-Si (mg/L)	Dissolved PO4-P (mg/L)	Total P (mg/L)	Dissolved NO2-N (µg/L)	NH4-N (µg/L)	NO3-N + NO2-N (µg/L)	Total N (µg/L)
07/25/13	No Zoo Initial	4.6	0.6	0.6	27.8	113.8	1551.6	2102.4
07/25/13	1X Initial	4.3	0.6	0.6	25.3	91.0	1589.1	2260.8
07/25/13	7.5X Initial	4.3	0.6	0.7	22.9	93.3	1439.7	2630.9
07/25/13	15X Initial	4.3	0.6	0.7	25.4	95.3	1373.1	2697.5
07/25/13	22.5X Initial	4.4	0.6	0.8	26.9	113.0	1524.4	3206.3
07/25/13	30X Initial	4.5	0.6	0.9	23.3	107.7	1638.6	3236.7
07/25/13	No Zoo Final	4.6	0.6	0.7	22.5	16.5	1495.8	2126.9
07/25/13	1X Final	4.3	0.5	0.6	23.2	7.0	1420.5	2102.1
07/25/13	7.5X Final	4.4	0.6	0.7	23.8	53.3	1374.7	2719.8
07/25/13	15X Final	4.5	0.6	0.7	23.9	80.1	1463.4	3065.7
07/25/13	22.5X Final	4.4	0.6	0.8	24.1	121.8	1564.0	3064.4
07/25/13	30X Final	4.4	0.6	0.8	28.3	159.4	1489.7	3109.4